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Skeletal Variants as an Indicator of Biological Effects of Environmental Contaminants

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16. ABSTRACT

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Several pesticides and a number of known teratogens were among the tested compounds.When necessary, toxicity tests were performed.

Eighty-eight variants were selected for the study, using CD-1 mice. Prenatal studies included fetal sdeletal parameters to provide comparability to teratogen studies.

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"SKELETAL VARIANTS AS AN INDICATOR OF BIOLOGICAL EFFECTS OF ENVIRONMENTAL CONTAMINANTS"

by

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FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The Health Effects Research Laboratory, Research Triangle Park, conducts a corrdinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. These studies address problems in air pollution, non-ionizing radiation, environmental carcinogenesis and the toxicology of pesticides as well as other chemical pollutants. The Laboratory participates in the development and revision of air quality criteria documents on pollutants for which national ambient air quality standards exist or are proposed, probides the data for registration of new pesticides or proposed suspension of those already in use, conducts research on hazardous and toxic materials, and is primarily responsible for providing the health basis for non-ionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of health care and surveillance of persons having suffered imminent and substantial endanagerment of their health.

This project uses an experimental design to attempt to develop a skeletal bariant assay system (SVAS) to pursue the possibility of detection of biological effects of in utero exposure to noxious substances.

F.G. Hueter, Ph.D.
Director,
Health Effects Research Laboratory

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ABSTRACT

The Skeletal Variant Assay System (SVAS) explores the possible detection of subtle biological effects of *in utero* exposure to noxious substances. This was attempted even in the absence of gross malformations, by an examination of a series of variations in development of adult skeletons.

Several pesticides and a number of known teratogens were among the tested compounds. When necessary, toxicity tests were performed.

Eighty-eight variants were selected for the study, using CD-1 mice. Fetal skeletal parameters were included in prenatal studies to provide comparability to teratogen studies.

This report was submitted in fulfillment of Grant Number R805420 by Weaton College under the sponsorship of the U. S. Environmental Protection Agency.

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INTRODUCTION

The project consisted of an attempt at developing a Skeletal Variant Assay System (SVAS) in the mouse whereby it might be possible to detect subtle biological effects of *in utero* exposure to noxious substances even in the absence of gross malformations, by an examination of a series of variations in development in adult skeletons.

The compounds tested included several pesticides and a number of known teratogens. Animals were CD-1 mice. Doses were chosen to include potentially teratogenic and no effect levels; they were administered by the most appropriate route and at appropriate times; choices were culled from published protocols wherever these were available. Toxicity tests were performed when necessary. Vehicle controls and Untreated controls were included in each series.

Eighty-eight variants were selected for study. They were all qualitative in nature to increase ease of typing, objectivity, and reliability. Most had previously been described in the literature (see Berry and Searle, 1963, Proc. Roy. Soc. London *140*: 577-615, and Berry, 1963, Genet. Res. *4*: 193-220 for descriptions of most of the variants). Most were polymorphic in CD-1 mice and thus what was measured was change in frequency of occurrence in response to treatment.

In many cases a prenatal study was also performed in order to provide comparability with traditional teratogen studies. A series of fetal skeletal parameters was included in the prenatal studies.

A series of adult skeletons of four-way outcross mice which had been selected for susceptibility and resistence to the teratogen trypan blue were available and were also examined for the SVAS variants as a separate confirming study.

A timetable of tasks performed during the tenure of the grant is presented as Table I.

METHODS

The experimental design for all compounds was very similar. Mice were purchased as needed from Charles River Breeding Laboratories (Wilm. MA) and maintained for, usually, three to six weeks before the start of the experiments, in shoebox-type cages, five animals of one sex per cage, at 72±2°F, relative humidity at 50±5%, on a light:dark regime of 18hr:6Hr, with Purina Mouse Chow and tap water available *ad libitum*. Groups of five females were mated with a single male at 4:00PM and checked for vaginal plugs the following morning. Mated females were isolated to experimental pens, with five females per pen, each from a different mating, receiving the same treatment. Each male contributed to different experimental groups. The order of the experimental pens was randomized on the shelves.

Females were isolated after their last treatment and bore and raised their litters in isolation. Litters were counted and weighed within one day of birth and examined for gross abnormalities. They were again examined, sexed, and weighed at weaning and allowed to remain together until sacrifice at 62±2 days of age. Litters were killed, identified with unique numbers, processed for alizarin staining of articulated skeletons, examined for a battery of 44 variants (see Table 3 for details), reprocessed for study of an additional battery of 44 variants in papain-digested, disarticulated skeletons (see Table 3) and stored. Details of specimen processing are presented in Beck, 1981, Teratology, Volume 21 (see Appendix E).

Frequencies of each variant in each experimental group were calculated and each experimental was compared with Untreated and Vehicle Control values for each variant. Of interest were the number of variants which differed in the experimental groups, the magnitude and direction of the differences, and the specific variants which were affected.

Statistical analysis initially involved both Mann-Whitney U tests on mean litter percent derived frequencies with significance being defined as $P<.05$ and t-tests on arcsin square-root transformed frequencies with significance being defined as $P<.01$ to compensate for Type I errors (Sokal and Rohlf, 1969, Biometry, W.H. Freeman, pp. 607-610). It was subsequently felt that the Mann Whitney test discarded potentially useful data and the t-test became the standard test of significance for the preliminary analyses.

In the prenatal studies fetuses were dissected on the 18th day of gestation, examined for gross malformations, weighed, and preserved in alcohol or formalin for alizarin staining. Fetal skeletons were studied for ossification of cervical centra, caudal vertebrae, number of metatarsals and metacarpals, number of phalangeal rows, and number of ribs and sternebrae.

All of the variants which occurred in these experiments were photographed as were additional representative specimens of interest, thus providing a permanent record. Macrophotography of most of the skeletal elements was accomplished with a Nikon F2 Photomic camera with a Micro lens and a bellows, using Kodak Plus-X film developed in Microdol-X. A green filter was used to increase contrast. Some specimens were photographed through a dissecting microscope with a Polaroid Camera on Type 47 film. A photographic atlas of the variants is presented in Figures 1-95.

The treatments are presented in Table 2. Doses were ultimately chosen which produced little or no maternal toxicity or morbidity, except for the 2,4,5-T study which was begun earlier.

RESULTS

Skeletal Variant Assay System

A complete, numbered list of the variants appears in Table 3, along with a complete analysis of the series of experiments with 2,4,5-T and Trifluralin, and references to descriptions of the variants. This table serves as an example, and that study is currently in press in Teratology, vol. 23, No. 1, 1981 (see Appendix E). Data from reproduction studies are presented in tabular form as "Vital Statistics" in Appendix A. Data from prenatal studies are presented in tabular form in Appendix B, along with explanations in the legend of each table. Data from the examination of the 88 characters in adult skeletons at 62±2 days of age (SVAS) are presented in tabular form as frequencies of occurrence, along with the number of animals in each experimental group, in Appendix C. SVAS data from several generations of maximally outbred mice (CBHA) which were crosses of C57BL/Gr, CBA/Gr, C3H/C-Hw, and A/Gr (see Beck, 1971, J. Exp. Zool. 177: 479-492 for detailed descriptions of the animals, mating scheme, and experimental protocols) are presented in Appendix D. These animals were selected for susceptibility (CBHA-S) and resistance (CBHA-R) to trypan blue teratogeneses and include an unselected control line (CBHA-C).

Table 4 presents a summary of significances, based on t-test analyses of arcsin-square root transformed frequencies when each character in each treated group is contrasted to the untreated (UNTD) control for that experiment and, separately, when each compound-treated group is contrasted with the appropriate vehicle (VEH) control, for all eleven series of experiments with CD-1 mice. One observation from this table is that 11 traits did not occur in any of the 11 series of experiments, except perhaps as single sporadic (unique) events (#2,4,5,12,13,31,34,39,40,41,85), 9 others occurred in only one (#1,7,24,28), two (#32,38,58) or three (#3,19) series. Thus 68 variants were useful in this assay. The number of characters which varied in each experiment and the number of variants which differed significantly from UNTD, VEH, or both, for each group in each experiment is presented in Table 5. Considering the number of variants affected, it is clear that prenatal exposure to 245-T, Trifluralin (TFL), Decamethrin (DMT), trypan blue (TB), acetazolamide (ACZM), or bromodeoxyuridine (BUDR), are all detectable at high dose levels, and not at no-effect levels, by examination of the postnatal skeletons, even in the absence of gross morphological abnormalities. Prenatal exposure to Captan, Thalidomide, cortisone (CORT), diphenylhydantoin (DPH), and probably Maneb, are not. For the latter group of compounds there are not any greater number of variants affected than are seen in untreated-vehicle treated comparisons. Other kinds of comparisons such as magnitude of differences, spectrum of variants affected, and cryptic malformations will be discussed later.

It became apparent (Tables 6 and 7) that different groups of untreated mice, purchased separately for each experimental series, did differ markedly from one another. Frequencies of each variant among untreated CD-1 mice in the first seven series of experiments are presented in Table C13.

Table C14 (data) and Table 8 (analysis) suggest that two different vehicle treatments applied to the same batch of animals (MBDM, ACZM-1, ACZM-2, BUDR-1 for example) typically do not differ greatly from each other in any of the experiments in which this analysis was performed. Table 8 further suggests that some of the significant differences between VEH and UNTD are due to handling effects, but that there may well be some systematic effects of vehicle treatment itself. Thus all results in Table 4 and elsewhere are contrasted both with VEH and with UNTD. One can argue that characters the frequencies of which differ from one or the other of UNTD or VEH, but not from both, are likely due to solvent or handling effects, or chance, given the number of characters and the variability in the system; characters which differ from both UNTD and VEH are likely a consequence of treatment with the compound.

Table 9 summarizes the significant findings which relate to the characters which are important indicators of subtle biological effects of treatment *in utero* with potentially noxious agents. Interfrontal (#14), Parted Frontals (#16), and Preoptic Sutures (#26) in the skull, Imperfect Transverse

Foramina (F.T.I.) of the Atlas (#47), Reduced Articular Processes of Thoracic Vertebrae (#68), 27-Presacral Vertebrae (#78), and Sacral Fusions (#79,80, 81) in the vertebral column, perhaps Carpal Fusions (#75) in the appendicular skeleton, and the occurrence of Fourteen Ribs (#70), are all sensitive indicators by virtue of the number of times their frequencies of occurrence differ significantly from both UNTD and VEH groups. Some characters, like #71-Malformed Ribs, #77-Lumbar Fusions, and #80-Fusions of Transverse Processes of Sacral Vertebrae appear to respond only to teratogenic doses of the compounds used. Others will respond to lower doses as well. Frequencies of occurrence as well as the magnitude of the differences in frequency from UNTD and from VEH are shown graphically for fourteen of the important variants in Figures 96-109. These figures clearly suggest that some of the variants respond dramatically to specific compounds - viz: #14 Interfrontal and #75 Carpal Fusions to TB and BUDR; #47 F.T.I. of the Atlas to TB; #20 Accessory Parietal and #78 27-Presacral Vertebrae (on day 8 only) to ACZM; #79 Fusions of Dorsal Processes of Sacral Vertebrae to 245-T and ACZM; #81 Fusions of the Vertebral Body of Sacral Vertebrae to ACZM (especially on days 9-11 treatment regime) and TB; #87 Fewer than 30 Caudal Vertebrae to 245-T, TFL and perhaps ACZM; and #88 Caudal Fusions to ACZM and TB. Others, like #16 Parted Frontals, #54 Accessory Transverse Foramina of the Cervical Vertebrae, #70 Fourteen Ribs, and #73 Malformed Sternebrae are affected by a broad spectrum of compounds and at several dose levels. Most of the treatment effects on the important variants involved an increase in frequencies of occurrence of the variants.

In many of the experimental series there were also variants which were singularly affected (Table 4). Thus, one sees #17 Fused Frontals and #18 Frontal Foramen Double strongly influenced only in 2,4,5-T treated animals. These form part of a strong cluster of frontal bone variants. This group (2,4,5-T) is also unique in a really major increase in absence of the prominent dorsal spine of the second thoracic vertebra (#62). Only in the TFL treatment is there an effect on #24 Foramen Palatinum Minus Posterior Double. Decamethrin is the only compound which affects #53 Foramina Transversaria Imperfecta of C3-C6 in an important way. Among the important effects of TB treatment, Dyssymphysis of the Atlas (#46) is markedly affected in both series and only in the trypan blue series. Malformations and dyssymphyses of the cervical vertebrae (#59) is seen only in these experiments and, interestingly, in the Captan treated group. Accessory Parietal (#20) and Abnormal Metoptic Roots (#27) are strongly affected only in the day 8 ACZM experiments, both series, and Lumbar Malformations and Fusions (#77) is strongly affected in both ACZM experiments on both day 8 and days 9-11 (also in the day 8 and day 9 treatment with BUDR in the second series only) and nowhere else. Fusion of the transverse processes of the sacral vertebrae (#80) shows a similar response to #77.

If one wanted to select a "quick and dirty" list of variants for a broadly based screen using post natal effects on the adult skeleton that would likely allow detection of prenatal exposure to a number of compounds, one could, based on these data, be reasonably confident in selecting #14 Interfrontal and #16 Parted Frontals in the skull, #47 Imperfect Transverse Foramina of the Atlas, #77 Lumbar Fusions, #78 Twenty Seven Presacral Vertebrae, and #79-81 Sacral Fusions in the vertebral column, and #70 Fourteen Ribs. While there are other variants which are useful indicators as well (see Table 9) the ones listed have the virtue of being easily and unambiguously ascertainable.

Of major concern is the reproducibility of these assays. Three sets of experiments (TB, ACZM, BUDR) were replicated. The raw data appear in Appendix C, important variants can be compared graphically in Figures 96-109, and Figures 110-112 present bar graphs of each set of replicated experiments, showing the difference from untreated of all 88 characters. Significant differences are indicated by stars. Figure 110 presents the Trypan Blue data. In general the agreement between the two series is excellent. Even when differences were non-significant they strongly showed similar patterns of variation in the two series. At the highest dose there were 13 variants (#14,15,33,46,47,54,59,71,75,81,86,87,88) which were significantly affected in both series, another 5 which were affected in the first series but not the second (#25,43,48,49,77) and 9 which were affected in the second series but not the first (#9,10,16,64,67,69,72,78,79). Table 10 presents those variants which differ importantly in the TB experiments. That is to say, these variants typically show significant differences from both Untreated and Vehicle, at least in the high dose group, in both experimental series.

Interestingly, Table C4 shows that among those traits which were significantly affected in several groups 8 were dose related in TB series 1 (#14,47,49,54,59,74,75,78) and 10 were dose related in TB series 2 (#14, 46,47,49,59,67,72,75,81,88). Strict dose relationships also occurred for several other traits which did not reach significance in each TB series. The large number of dose related responses suggests the usefulness for detecting subtle effects of treatment. The trypan blue experiments, with the large number of dose levels was designed to reveal this type of response.

Figure 111 and Table 11 present the same analysis for the acetazolamide experiments. There was even better agreement between the two ACZM series in the day 9-11 treatments than in the TB experiments, with 13 variants being significantly different from Untreated in both series (#21,33, 45,52,70,73,74,77,79,80,81,87,88), only two being significantly affected in series one only (#22,47), and 5 affected in series two only (#16,54,68,72, 83) in the high dose group. The agreement between the two series in the day 8 treatment was less close. Eight variants were significantly different from Untreated in both series (#16,20,27,68,70,77,78,79), whereas 9 variants were significantly affected in series two only (#10,14,26,30,48,59, 67,69,82) and 4 variants which were affected in the first series only #54, 75,80,81). Table 11 presents those variants which differ importantly in the ACZM experiments. Although the list consists of 21 variants, some are only important on day 8 (#20,26,27,78), others only in the day 9-11 treatment (#33,45,47,52,73,74,83,87,88), and still others are seen to be importantly affected by both treatment regimes (#16,54,68,70,77,79,80,81).

Agreement between the two series of BUDR experiments (Figure 112) was less close than in either the TB or ACZM series. Still, 7 characters showed significant differences from UNTD in both series of BUDR-day 7 high dose (#14,26,47,59,70,74,78), though in one case (#47) the deviation was in opposite directions in the two series. Six additional variants differed in series one only (#20,37,63,68,71,82) and five differed in series two only (#29,42,52,55,67). In the high dose day 8 groups, 8 variants differed from

UNTD in both series (#26,36,37,70,71,73,74,78), with 4 others differing in series one only (#14,49,56,82) and 6 differing in series two only (#48,52,61,67,68,77). The low dose showed little effect, especially on day 7. The group of variants which showed important differences (Table 12) numbers only 12, and perhaps also #47 F.T.I. of the Atlas. Of the 12, five are importantly affected both on day 7 and day 8 (#26,70,71,74,78) with two more clearly affected on day 7, but ambiguously affected on day 8 (#68,82); four variants are importantly affected on day 7 only (#14,20, 48,59) and one on day 8 only (#73). BUDR, series 2 was the last set of experiments completed, and has not been reported previously in detail. Table 13 presents the usual analysis of these data. The addition of the day 9 treatment group is interesting in that it reveals a shift in the location of affected variants over time. In the day 7 treatment significant variants are fairly evenly distributed, there is a suggestion of a posteriad shift in the day 8 treatment group, and in the day 9 treatment only 3 of the 13 affected variants occur in the skull with most of the remainder being in the thorax and lumbo-sacral areas.

Prenatal Studies

The treatment regimes for the prenatal studies were the same as for the postnatal skeletal studies and are described in Table 2. The data appear in Appendix B. There were somewhat fewer, though still a significant proportion, of abnormalities in the trypan blue experiments (Table B2) than have been reported with other stocks of mice, but mortality was comparable to published reports. A substantial number of tail kinks did appear later, in the post natal experiments. Diphenylhydantoin (Table B6) must be considered essentially non-teratogenic and non-embryotoxic in these experiments. Maneb (Table B1) teratogenicity was borderline, but the compound produced significant ($P<.001$) prenatal mortality. Acetazolamide (Table B3), bromodeoxyuridine (Table B4) and cortisone (Table B5) produced results which are comparable to those reported in the literature. Ectrodactyly was common in day 9-11 ACZM; exencephaly with or without micrognathia and exophthalmia, and polydactylism were the common defects in the BUDR experiments; cleft palate and tail kinks were the preponderant malformations in the CORT experiments.

All of the fetuses were examined for a number of variations of the skeleton detectable at 18 days of gestation including the extent of the ossification of the centra of the cervical vertebrae and of the caudal vertebrae (Table B7), the number of ribs and ossification of sternebrae (Table B8), and extent of ossification of the bones of the hands and feet (Table B9).

For several of the compounds (BUDR, ACZM, TB) the proportion of individuals in which not all of the cervical centra showed ossification was substantially and significantly increased by a teratogenic dose. At the high doses in five of the six series (not in BUDR) the first cervical vertebra was most often affected and the seventh was least often affected. Caudal ossification was also reduced by treatment in all series except TB, based on frequency of individuals with six or fewer ossified caudal vertebrae. For a number of treatment regimes (BUDR, ACZM on day 8, TB at intermediate levels only, Maneb) the frequency of occurrence of 14 ribs was increased, and the proportion of 14 rib individuals with asymmetric expression was decreased. Sternebrae ossification was not affected in a systematic way. There appeared to be a reduction in ossification of metatarsals in several series of experiments (the day 9-11 ACZM is largely an artifact of the ectrodactyly occasioned by this treatment), but in no cases do the differences reach statistical significance.

Four-Way Outcross (CBHA) Skeletons

The decision to examine these specimens, despite the fact that they were fragmentary series and of different ages than the CD-1 mice studied, was the observation of great variability among separately purchased groups of untreated CD-1 animals. The four-way outcross CBHA mice were maintained by a strict regime of maximal outbreeding rather than being random bred, and were constituted from four inbred strains rather than having an unknown ancestry as in CD-1. Frequency data for generations (G) 2, 3, 6, 7, and 9 of the CBHA are presented in Tables D1-D6; comparisons across generations of untreated, unselected (U, or CBHA-C) animals, treated groups of CBHA-C (C, or CBHA-CT), animals selected for resistance (R, or CBHA-R) and susceptibility (S, or CBHA-S) to trypan blue teratogenesis are presented in Tables D7-D10 respectively. Tables D11-D13 present differences in frequency between untreated and S, R, and C respectively. Table 14 presents a summary of significant differences between CD-1 and the initial foundation, generations (G1, G2) of CBHA as well as differences in each CBHA generation from a variety of comparisons.

Comparing CBHA G1 and G2 with CD-1 (Table 14) reveals, unsurprisingly, that they are very different. G1 and G2 are, however, very similar to each other. Among untreated CBHA-C across four generations (Table D7), only 8 of the 88 characters show any significant ($P<.01$) variation based on a chi-square test of homogeneity (#10,30,33,51,52,66,74,80); 23 of the characters do not vary in any of the untreated series. Thus several groups of untreated animals are much more similar to one another than are different groups of CD-1. Results from treatment of subsamples of the unselected line show a similar consistency. Only 11 characters show significant variation across the 3 generations for which data are available (#8,10,21,22,29,35,44,51,52,76,80). Thus the response of unselected animals to trypan treatment is also reasonably consistent over generations. Responses of the selected lines are not relevant to the present report. Given the great variability of untreated CD-1 mice, it is essential to examine a series of untreated specimens for each separately purchased group of animals. The use of inbreds or other genetically known stocks might therefore be more time efficient.

SUMMARY AND CONCLUSIONS

Skeletons of CD-1 mice were examined in a systematic way at 62±2 days postnatal for a series of 88 normally occurring variations. Subjects had been exposed *in utero*, by treatment of their dams, to one of a group of pesticides - Trifluralin (TFL), 2,4,5-T (245-T), Captan, Maneb, Decamethrin (DMT); or known teratogens - Thalidomide, trypan blue (TB), acetazolamide (ACZM), bromodeoxyuridine (BUDR), diphenylhydantoin (DPH), cortisone (CORT), at both high and no-effect levels. Frequencies of occurrence of each variant in each treatment group were contrasted with vehicle (VEH) Control and Untreated (UNTD) values. Sixty-eight of the skeletal variants were useful in these studies; the others occurred rarely or not at all.

Prenatal exposure to 2,4,5-T, TFL, DMT, TB, ACZM, and BUDR could be detected by the existence of substantial numbers of skeletal variants the frequencies of which were significantly different from VEH and UNTD controls, at the higher dose levels. Prenatal exposure to Captan, Thalidomide, cortisone, diphenylhydantoin, and probably Maneb, could not be detected by this criterion. This suggests that the Skeletal Variant Assay System (SVAS) is a sensitive, yet not indiscriminate, method for detecting prenatal exposure to a variety of noxious substances, even in the absence of gross malformations. Considering the particular variants which were affected, it would appear that a smaller subgroup could serve as a reasonable screen for detecting subtle biological effects, by virtue of their having responded in a large number of the experimental series. These include the presence of an interfrontal bone, the existence of parted frontals, and preoptic sutures, in the skull; imperfect transverse foramina (F.T.I.) of the atlas, reduction of articular processes of the thoracic vertebrae, occurrence of 27 rather than the usual 26 presacral vertebrae, and sacral fusions, all in the vertebral column, perhaps carpal fusions, in the appendicular skeleton; and the occurrence of 14 ribs.

Some variants were only affected by teratogenic or high dose levels. These included malformations of the ribs, fusions in the lumbar vertebrae and in the transverse processes of the sacral vertebrae. Some of these might be considered cryptic malformations. In most of the series there were variants which were enormously affected only by one or two compounds. These included Interfrontal and Carpal Fusions in TB and BUDR experiments, F.T.I. of the atlas by TB, Accessory Parietal and 27 presacral vertebrae (on day 8 only) by ACZM, fusions of dorsal processes of sacral vertebrae by 2,4,5-T and ACZM, fusions of the vertebral body in sacral vertebrae by ACZM (especially in the days 9-11 treatment) and by TB, the occurrence of fewer than 30 caudal vertebrae in the 245-T, TFL and perhaps ACZM treated groups, and caudal fusions by ACZM and TB. Most of the treatment effects on the important variants in all studies resulted in increases in frequencies of occurrence.

In many of the experiments there were also variants which were uniquely affected. These included: Fused Frontals, Frontal Foramen Double and a really major increase in absence of prominent dorsal spine of the second thoracic vertebra in 245-T, doubling of the posterior foramen minus in the palatal bone by TFL, F.T.I. of C3-C6 by DMT, dyssymphysis of the atlas, malformations and dyssymphyses of the cervical vertebrae by TB, and the latter also in Captan treated animals, Accessory Parietal and Abnormal Metoptic Roots by ACZM on day 8, lumbar fusions and malformations in both ACZM regimes and in day 9 BUDR treatments.

Replication of experiments with TB, ACZM, and BUDR resulted in good to excellent agreement between the two series. Considerations of spectrum and magnitude of response, and idiosyncratic or specific responses, add strength to the ability of the SVAS to detect prenatal exposure to 245-T, TFL, DMT, TB, ACZM, and BUDR.

Prenatal studies with most of the compounds were in essential agreement with published results; DPH was neither teratogenic nor fetotoxic in these studies and was also negative in the SVAS; cortisone, although it had no effect on the skeletons of survivors, was a potent cleft palate producer. From the prenatal studies there was also the suggestion that ossification of the centra of the cervical vertebrae and of caudal vertebrae might be indicative of exposure to high doses of all or nearly all of the compounds.

Finally, examination of available skeletons from adult mice in several generations of a four-way outcross mouse population selected for susceptibility or resistance to trypan blue teratogenesis as well as an unselected line revealed better agreement across generations for each line that was seen in different batches of CD-1 mice. The variability of different "batches" of CD-1 mice require an examination of untreated animals in each group of animals purchased.

Table 1. Timetable of Tasks and Milestones.

First Year

	1977 October	November	December	1978 January	February	March	April	May	June	July	August	September
Mating				-(MBDM)--	--------MBDM	------	--------		--------	-TRBL1-	---------	
Treatment				-----(MBDM-	--	------MBDM	-----	--------	---	-----TRB1	------	
Prenatal Study								--MBDM--		-----TRBL1	------	
Litters					-(MBDM)--	---MBDM	----			-------	-TRBL1--	
Weaning						-(MBDM)	--------	MBDM---	------		----------	-TRBL1----
Kills								--------	MBDM--	-------		--TRBL1----
Alkali Digestion								--------	MBDM--	------		--TRBL1----
Alizarin Staining								--------	MBDM--	-----		--TRBL1----
Clearing									-------	-MBDM--		--TRBL1----
Examination: Articulated									-----MBDM	-----		
Papain Digestion									-----MBDM	-----		
Examination Disarticulated										--MBDM-		
Data Analysis										--MBDM-		
Report				-			-			--		
Manuscript Preparation												

Laboratory set up, acquisition of supplies, equipment, animals. Hiring and instruction of technicians and animal caretaker.

Blizzard required reestablishing initial set of experiments.

Table 1. Continued

Second Year

	1978 October	November	December	1979 January	February	March	April	May	June	July	August	September
Mating		--ACZM1--		---BUDR1------			-TB2-	--ACZM2---		-----CORT-		
Treatment		--ACZM1-		----BUDR1-----			-TB2--	---ACZM2----		-----CORT-		
Prenatal Study		---ACZM1-			------BUDR1--						----CORT-----	
Litters		--ACZM1--			-----BUDR1-----		--TB2--		--ACZM2--		----CORT---	
Weaning			--ACZM1--			---BUDR1---		--TB2-		--ACZM2---		---CORT----
Kills	--TRBL1--			---ACZM1--			--BUDR1-		---TB2--		--ACZM2----	
Alkali Digestion	---TRBL1--			---ACZM1---			--BUDR1--		---TB2---		---ACZM2----	
Alizarin Staining	-----TRBL1--				--ACZM1---		---BUDR1---		--TB2----		-----ACZM2-----	
Clearing	------TRBL1---		-G367--	--G9--	--ACZM1---			---BUDR1--	---TB2---		-----ACZM2-----	
Examination: Articulated	---TRBL1-----		-G367---	- G9--	---ACZM1------			--BUDR1--		-TB2----	-----ACZM2-----	
Papain Digestion	---TRBL1------		-G367-	-G9---		----ACZM1----		---BUDR1----		--TB2--	-----ACZM2-----	
Examination Disarticulated		----TRBL1----	-G367-	-G9---		-----ACZM1--		---BUDR1----		--TB2--	---ACZM2-------	
Data Analysis			-T1 -G367-	-T1	--G9-		-A1--		-T1,A1,B1,G3,6,7,9,T2			-A2-
Report	--			--			--			--		
Manuscript Preparation									--Proposal, Photo Atlas, MS-------			

Table 1. Continued

	Third Year											
	1979			1980								
	October	November	December	January	February	March	April	May	June	July	August	September
Mating			----DPH------		-----BUDR2---							
Treatment	CORT-		----DPH-----		----BUDR2------							
Prenatal Study	CORT-			-DPH--								
Litters	CORT-		--DPH--------			----BUDR2---						
Weaning	--CORT-			---DPH-------			--BUDR2--------					
Kills		---CORT---			---DPH-------			--BUDR2-------				
Alkali Digestion		---CORT---			---DPH---------			---BUDR2---------				
Alizarin Staining			---CORT---		---DPH---------			---BUDR2---------				
Clearing			---CORT---		--DPH---------			---BUDR2---------				
Examination: Articulated			----CORT------		-DPH----------				------BUDR2------------			
Papain Digestion			----CORT---		-DPH----------				------BUDR2------------			
Examination Disarticulated			--CORT----			---DPH--------			----BUDR2---------------			
Data Analysis				-CORT-			-DPH--					--BUDR2----
Report	--			--			-			--		
Manuscript Preparation										Draft Final Report	Graphs and Figures	MS

Table 1. Legend

MBDM = Maneb Decamethrin experiments

TRBL1 = T1 = First series of Trypan Blue experiments

ACZM1 = A1 = First Acetazolamide series

BUDR1 = B1 = First Bromodeoxyuridine series

TB2 = T2 = Second Trypan Blue series

ACZM2 = A2 = Second Acetazolamide series

CORT = Cortisone experiments

G3,6,7,9 = Generations of four-way outcross (C57BL, CBA, C3H, A) skeletons from earlier selection experiments for sensivity and resistance to trypan blue

MS = manuscript

DPH = Diphenylhydantoin experiments

BUDR2 = Second series of Bromodeoxyuridine experiments

Table 2. Experimental Protocols

Compound(s) Tested	Exptl. Groups	Abbreviation[a]	Treatment	Delivered Volume	Route	Duration of Treatment (DPC)	Litters	Individual	Comment
Trifluralin/2,4,5-T		(TFLT)							b
	1	UNTD	None				10	94	
	2	VEH	Corn Oil	3.3ml/kg/day	gavage	6-15	10	95	
	3	245T-L	20mg 2,4,5-T/kg/day in Corn Oil				11	93	
	4	245T-H	100mg 2,4,5T/kg/day in Corn Oil				11	95	
	5	TFL	1.0gm Trifluralin/ kg/day in Corn Oil				11	92	
Thalidomide/Captan		(TCAP)							c
	1	UNTD	None				11	92	
	2	VEH	0.5% Carboxymethyl Cellulose (CMC)	3.3ml/kg/day	gavage	6-15	10	91	
	3	Thal-L	20mg Thalidomide/ kg/day in CMC				11	92	
	4	Thal-H	100mg Thalidomide/ kg/day in CMC				11	91	
	5	Capt	100mg Captan/kg/ day in CMC				11	92	
Maneb/Decamethrin		(MBDM)							d
	1	UNTD	None				10	104	
	2	CMC	1.0% Carboxymethyl	3.3ml/kg/day	gavage	6-15	10	101	
	3	MNB	1200mg Maneb/kg/ day in CMC				13	102	
	4	COC	Corn Oil Control				10	100	
	5	DMT	12mg/kg/day in Corn Oil				9	102	

Table 2. Continued.

Compound(s) Tested	Exptl. Groups	Abbreviation[a]	Treatment	Delivered Volume	Route	Duration of Treatment (DPC)	Litters	Individual	Comment
Trypan Blue		(TRBL-1,TRBL-2)							e,f
	1	UNTD	None				11/12	100/100	
	2	VEH	0.9% Sodium Chloride (Saline)	0.25cc/day	i.p.	7-9	12/9	100/97	
	3	.037T	0.375%(w/v) Trypan Blue/mouse/day, in Saline				12/12	96/75	
	4	.075T	0.75%(w/v) Trypan Blue/mouse/day, in Saline				11/12	86/75	
	5	0.15T	0.15%(w/v) Trypan Blue/mouse/day, in Saline				12/14	79/65	
	6	0.30T	0.30%(w/v) Trypan Blue/mouse/day, in Saline				17/16	71/100	
Acetazolamide		(ACZM-1,ACZM-2)							f,g
	1	UNTD	None				11/12	98/100	
	2	VEH-8	Distilled water (H_2O)	10.0ml/kg	i.p.	split dose, 7AM,3PM,12M, day 8	9/13	100/100	
	3	ACZM-L8	200mg/kg, in H_2O				9/8	99/89	
	4	ACZM-H8	1.0gm/kg, in H_2O				11/14	96/98	
	5	VEH-9-11	H_2O			split dose, 7AM,3PM,12PM, on each of days 9,10,and 11	11/11	97/83	
	6	ACZM-L9-11	200mg/kg/day				10/11	88/99	
	7	ACZM-H9-11	1.0gm/kg/day				10/10	100/80	

Table 2. Continued.

Compound(s) Tested	Exptl. Groups	Abbreviation[a]	Treatment	Delivered Volume	Route	Duration of Treatment (DPC)	Litters	Individual	Comment
Bromodeoxyuridine (BUDR-1,BUDR-2)									h
	1	UNTD	None				8/10	98/97	
	2	VEH-7	Distilled water (H_2O)	15.0ml/kg	i.p.	7	9/10	97/99	
	3	BUDR-L7	60mg/kg, in H_2O				9/10	98/97	
	4	BUDR-H7	300mg/kg, in H_2O				10/12	100/87	
	5	VEH-8	H_2O			8	8/11	98/96	
	6	BUDR-L8	60mg/kg, in H_2O				11/10	99/98	
	7	BUDR-H8	300mg/kg, in H_2O				10/21	99/100	
	8	VEH-9	H_2O			9	/10	/93	
	9	BUDR-L9	60mg/kg, in H_2O				/10	/96	
	10	BUDR-H9	300mg/kg, in H_2O				/12	/92	
Cortisone		(CORT)							i
	1	UNTD	None				10	94	
	2	VEH	0.85% Sodium Chloride (NaCl)	0.1cc/day	i.m.	11-14	11	98	
	3	.15 Cort	.1563mg Cort/mouse/day, in NaCl				10	98	
	4	.3 Cort	.3125mg Cort/mouse/day, in NaCl				10	97	
	5	.6 Cort	.625mg Cort/mouse/day, in NaCl				10	98	
	6	1.2 Cort	1.25mg Cort/mouse/day, in NaCl				16	99	

Table 2. Continued.

Compound(s) Tested	Exptl. Groups	Abbreviation[a]	Treatment	Delivered Volume	Route	Duration of Treatment (DPC)	Litters	Individual	Comment
Diphenylhydantoin		(DPH)							j
	1	UNTD	None				10	98	
	2	VEH	0.05% Sodium Hydroxide (NaOH)	3.3mg/kg/day	i.p.	11-13	11	100	
	3	6.25DPH	6.25mg DPH/kg/day, in NaOH				11	98	
	4	12.5 DPH	12.5mg DPH/kg/day, in NaOH				11	98	
	5	25 DPH	25.0mg DPH/kg/day, in NaOH				10	99	
	6	50 DPH	50mg DPH/kg/day, in NaOH				10	95	

Table 2. Legend

a. These abbreviations are used in several tables.

b. This experiment was actually performed before the inception of the grant, but additional variants were examined, data analysis was performed and a manuscript was prepared during the tenure of the grant.

c. This experiment was also performed before the inception of the grant; additional variants were examined, and additional data analysis performed. Since the results were essentially negative, no manuscript was prepared.

d. Initially a toxicity study was performed with Maneb to establish an appropriate treatment level. At 1500 mg/kg there was substantial mortality and even at 1200 mg/kg some maternal mortality occurred. Compounds were obtained from EPA-HERL-ETD, Research Triangle Park, N.C.

e. The two series of experiments were identical, performed about ten months apart. The highest concentration was made up directly, and others were made by dilution. It is traditional to deliver a uniform dose irrespective of weight of the dam. Compound was manufactured by Allied Chemical Co. (lot #0465157)

f. The second set of trypan blue experiments and Acetazolamide experiments were performed on the same group of CD-1 mice, nearly simultaneously, and shared a common Untreated group.

g. Acetazolamide was graciously provided by Lederle Laboratories, and was the sodium salt, Diamox ®, a product of American Cyanamid. The two series of experiments were essentially identical replications performed approximately six months apart.

h. Bromodeoxyuridine was purchased from Sigma Chemical Company. The two series of experiments were performed about ten months apart and consisted of the same dosing regime, except that only in the second series were day 9 treatment groups included.

i. Cortisone acetate was obtained from Calbiochem, Inc. A preliminary toxicity study revealed that 2.5 mg/mouse/day was too deleterious; 86.3% of the relatively small number of fetuses surviving to 18DPC had cleft palate. Only 21.3% of fetuses whose dams received 1.25mg/mouse/day showed cleft palate, and this was selected as the high dose for the experimental series. Lower doses were made by dilution of the high dose. Injections were made into the upper muscle mass of the hind leg, alternating legs during the four days of treatment.

j. Diphenylhydantoin was graciously provided as the sodium salt, Phenytoin Sodium ®, by Warner-Lambert Co. The high dose was made up each day as needed, and the lower doses made by dilution.

Table 3. Frequencies of Occurrence of Skeletal Variants Among CD-1 Mice and Significant Differences in Frequencies Among Experimentals Contrasted with Untreated or Vehicle Treated Groups

				Frequencies (%)					Significant Differences: Compared With Untreated				Compared With Vehicle			Exclusive of Solvent Effects		
Character Number	Reference	Variant Number	Character Name N =	UNTD 94	VEH 95	245T-L 93	245T-H 95	TFL 92	VEH	245T-L	245T-H	TFL	245T-L	245T-H	TFL	245T-L	245T-H	TFL
1A	t	N	Short Snout															
2A	t	N	Bent Nose															
3D		N	Fused Nasals															
4D		N	Nasal-Turbinal Fusion															
5D		N	Nasal Fused to Skull															
6D	d	1.	Preorbital Foramen Double	44.7	50.5	52.7	28.4	43.5			o			*			*	
7D	a	2.	Preorbital Foramen Absent	1.1	1.1	0	5.3	0										
8D	c	3.	Maxillary Foramen I Double	94.7	94.7	83.9	94.7	93.5		o			o					
9D	c	4.	Maxillary Foramen I Absent	1.1	3.2	2.2	0	3.3						o				
10D	c	5.	Maxillary Foramen II Double	40.4	17.9	24.7	23.2	17.4	*	o	o	*†						
11D	c	6.	Maxillary Foramen II Absent	4.3	18.9	7.5	18.9	25.8	*		*	*	o					
12D	c	N	Lachrymal-Maxillary Fusion															
13D		N	Jugal Not Fused															
14A	v	7.	Interfrontal	3.2	8.4	3.2	20.0	6.5			*†			o			*	
15A	v	8.	I.F.-Frontal Fusion	0	2.1	1.1	2.1	0	o		o				o			
16A	v	9.	Parted Frontals	23.4	24.2	10.8	50.5	46.7		o	*†	*†	o	*†	*		*	*
17A	g	10.	Fused Frontals	0	0	0	13.7	1.1			*†			*†			*	
18A	d	11.	Frontal Foramen Double	0	0	0	4.2	0			*			*			*	
19A	a	12.	Frontal Foramen Absent	6.4	3.2	2.2	5.3	6.5										
20A	a	13.	Accessory Parietal	1.1	3.2	2.2	8.4	4.3			*							
21D	d	14.	Foramen Palatinum Major Double	24.5	24.2	30.1	32.6	32.6										
22D	a	15.	Foramen Palatinum Minus Anterior Double	75.5	69.5	69.9	71.6	69.6										
23D	c	16.	Foramen Palatinum Minus Anterior Absent	8.5	10.5	5.4	23.2	15.2			*			o				
24D	a	17.	Foramen Palatinum Minus Posterior Double	1.1	1.1	1.1	0	13.0				*			*			*
25D	c	18.	Foramen Palatinum Minus Posterior Absent	8.5	9.5	7.5	4.3	1.1				*			*†			*

Table 3. (continued)

26D	w	19.	Preoptic Sutures (Presphenoid)	13.8	30.5	19.4	46.3	31.5	*		*†	*†		o				
27D	w	20.	Abnormal Metoptic Roots (Presphenoid)	51.1	46.3	34.4	49.5	47.8		o								
28D	g	U	Pre-Basisphenoid Fusion	U														
29D	e	21.	Foramen Sphenoidale Mediale Present	57.4	48.4	50.4	45.3	63.0							o			
30D	e	22.	Posterior Border of the Palatine: Anteriad Suture	76.6	77.9	72.0	92.6	82.6			*			*			*	
31D		N	Squamosal-Frontal Fusion															
32D	u	N	Squamosal-Parietal Fusion															
33D	e	23.	Foramen Ovale Single	36.2	37.9	44.1	49.5	62.0				*†			*†			*
34D	e	N	Foramen Ovale Open Posteriorly															
35D	c	24.	Foramen Infra Ovale Double	36.2	22.3	23.9	45.7	28.4	o					*				
36D	a	25.	Foramen Infra Ovale Absent	1.1	1.1	0	6.3	1.1			o			o				
37D	c	26.	Foramen Pterygoideus Double	2.1	3.2	2.2	9.6	7.9			o							
38D	e	27.	Processus Pterygoideus Absent	0	1.1	1.1	0	1.1										
39D	u	N	Occipital-Periotic Fusion															
40D	g	N	Interparietal-Occipital Fusion															
41D	d	N	Basisphenoid-Basiooccipital Fusion															
42A	d	28.	Fenestra Flocculi Absent	5.3	12.6	9.7	7.4	15.2				o						
43D	e	29.	Foramina Hypoglossi Double (Occipital)	89.4	92.6	94.6	92.6	88.0										
44D	c	N	Mandibular Foramen Double															
45A	e	30.	Accessory Mental Foramen	4.3	10.5	8.6	10.5	8.7										
46A	i	31.	Dyssymphysis Atlas (C.1)	1.1	1.1	1.1	0	1.1										
47D	s	32.	Foramina Transversaria Imperfecta Atlas (C.1)	8.5	7.4	2.2	60.0	6.5		o	*†			*†			*	
48A	i	33.	Dyssymphysis Axis (C.2)	3.2	3.2	2.2	28.4	4.3			*†			*†			*	
49D	s	34.	Foramina Transversaria Imperfecta Axis (C.2)	3.2	1.1	11.8	48.4	1.1		o	*†		*	*†		*	*	
50D	i	35.	Spinous Process C.3	0	0	0	0	2.2				o			o			
51A	u	36.	Arch Foramen C.4 Absent	31.9	40.0	15.1	6.3	19.6		*	*†		*†	*†	*†	*	*	
52A	u	37.	Arch Foramen C.5 Present	6.4	3.2	7.5	22.8	2.2			*			*†			*	
53D	i	38.	Foramina Transversaria Imperfecta C.3-C.6	7.4	22.1	8.6	27.4	2.2	*		*		*		*†			

Table 3. (continued)

54D	x	39.	Accessory Transverse Foramina C.3-C.6	57.4	49.5	59.1	60.0	78.3				*			*†	*
55A	i	40.	Tubercula Anteriori C.6 Absent	1.1	0	0	1.1	0								
56A	i	41.	Tubercula Anteriori C.6 Inflexed	1.1	1.1	0	1.1	0								
57A	i	42.	Tubercula Anteriori C.6 Dystopic	0	0	1.1	1.1	0								
58A	d	U	Tubercula Anteriori C.6 Accessory					U								
59A	i,s	43.	Malformations (Dyss/Fus) C.3-C.7	0	0	2.2	0	2.2		o			o		o	
60D	d	44.	Foramina Transversaria C.7	54.3	52.6	64.1	56.8	45.7				o				
61A	i,t	45.	Malformations (Dyss/Fus) Th.1-Th.2	1.1	2.1	1.1	1.1	3.3								
62A	i	46.	Spinous Process Th.2 Absent	3.2	0	7.5	45.3	2.2	o		*†		*	†*	o	*
63A	i	47.	Spinous Process Th.2 Reduced	0	6.3	2.2	10.5	6.5	*†	o	*	*†				
64A	a	48.	Spinous Process Th.2 Jointed	10.6	2.1	8.6	2.1	4.3	o		o		o			
65A	i	49.	Spinous Process Th.2 Dystopic	1.1	0	2.2	1.1	0					o			
66A	i	50.	Spinous Process Th.2 Accessory	0	2.1	0	0	2.2	o			o	o	o		
67A	s	51.	Arch Foramina Thoracic Vert.	75.5	64.2	78.5	66.3	70.7					o			
68A	p	52.	Reduced Articular Processes-Thoracic Vert.	10.6	13.5	24.7	14.7	27.2		o		*†			o	
69A	g	53.	Malformations (Dyss/Fus) Th.3-Th.14	0	3.2	2.2	1.1	2.2	o	o		o				
70A	h,r	54.	Fourteen Ribs	22.3	24.2	32.3	30.5	58.7				*†			*†	*
71A	n	U	Malformed Ribs				U									
72A	k	55.	Fused Sternebrae	50.0	41.1	47.3	63.2	45.7						*		
73A	k	56.	Malformed Sternebrae	71.3	69.5	67.7	69.5	78.3								
74	x	57.	Fossa Olecrani Perforata (Humerus)	55.3	45.3	58.1	31.6	60.9			*				o	
75A	b,f,m	58.	Carpal Fusions	10.6	15.8	14.0	5.3	23.9				o		o		
76D	q,s	U	Sacralized L.6			U										

Table 3. (continued)

77A		U	Lumbar Fusions/Malf															
78A	h	59.	27-Presacral Vertebrae		U													
79A	q,s	60.	Sacral Fusions Dorsal Processes	0	0	3.2	2.1	0		o	o		o	o				
80A		61.	Sacral Fusions Transverse Processes	23.4	21.2	15.1	40.0	29.3			o			*			*	
81A		62.	Sacral Fusions Vertebral Body	1.1	0	0	0	1.1										
82A	q	63.	Caudalized S.3	1.1	2.1	0	2.1	4.3										
83D	k	64.	Foramina Acetabuli (Innominate)	3.2	18.9	17.2	3.2	6.5	*	*				*	*			
84D	i,k	65.	Dyssymphysis Ischio-Pubica	31.9	28.4	35.5	32.6	33.7										
85D	d	N	Pubic Fusion	1.1	1.1	2.2	3.2	2.2										
86A	b,f,m	N	Tarsal Fusion															
87A		66.	Fewer Than 30 Caudal Vert.	19.1	25.3	36.6	58.5	42.4		*	*	*		*	*		*	
88A		67.	Caudal Fusions	1.1	1.1	0	0	1.1										
Number of Significant Differences								t-test	6	3	19	12	4	16	9	2	14	6
								Mann-Whitney U	1	0	9	7	1	8	8	2	8	4

Legend for Table 3.

Character Number = An anterior-posterior listing of the 88 characters investigated. A = Examined in articulated skeletons; D = Examined in disarticulated skeletons

Reference - Sources of description of each character

For complete citation see Beck, S.L. 1981, Teratology (in press).

a. New Variant, described here
b. Beck and Smith '72
c. Berry '63
d. Berry and Searle '63
e. Deol '55
f. Deol '61
g. Deol and Trusolve '57
h. Green '41
i. Grüneberg '50
j. Grüneberg '51
k. Grüneberg '52
m. Grüneberg '56
n. Grüneberg '63
p. Johnson '68
q. Runner '53
r. Palmer '72
s. Searle '54a
t. Searle '54b
u. Searle '54c
v. Truslove '52
w. Truslove '54
x. Weber '50

Variant Number = An anterior-posterior listing of those character which vary in these experiments. Variants are defined as characters which occur in more than one of the 469 individuals examined. N = Null class - character not seen in any specimen; U = Unique observation - character seen in a single specimen. The letter also appears in the column in which the unique observation occurred.

Character Name = Names used to describe characters. Most are the terminology of Bateman (1954), Berry (1963), or are described in the text.

C = Cervical, Th = Thoracic, L = Lumbar, S = Sacral Vertebrae
Dyss = Dyssymphysis
Fus = Fusion
Vert = Vertebrae, Malf = Malformation

N = Number of Specimens in each group

Frequencies = Percentage of specimens in each group which manifest each variant.

UNTD = Untreated, unhandled controls

VEH = Corn oil vehicle control. Vehicle control dams were intubated intragastrically with 3.3 ml/kg body weight of corn oil on days 6-15 of gestation.

245T-L = Low dose (negative control) of 2,4,5-T. Dams were intubated with 3.3 mg/kg/day of corn oil containing 20 mg/kg/treatment of 2,4,5-T on days 6-15 of gestation.

245-H = High dose (positive control) of 2,4,5-T. Dams were intubated with 100 mg 2,4,5-T/kg body weight/days 6-15 of gestation in 3.3 ml/kg/treatment of corn oil

TFL = Trifluralin (unknown compound). Dams were intubated with 1.0 gm Trifluralin/kg body weight in 3.3 ml/kg corn oil, warmed to 38-40°C, on each of days 6-15 of gestation.

Significant Differences

Tests of significance, contrasting each treatment group with UNTD, and each compound-treated group with VEH (the UNTD-VEH comparison is identical with a VEH-UNTD comparison and is not repeated). The statistical analysis includes t-tests on arcsin-square root transformed frequencies (Sokal and Rohlf 1969, pp. 607-610) where $t = \text{arcsin}\ p_1 - \text{arcsin}\ p_2 / \sqrt{820.8(1/n_1 + 1/n_2)}$, significance being defined as $P < .01$, and the individual mouse is the experimental unit. * = $P < .01$; o = $P < .05$, presented for completeness. Also represented in this group of columns is the results of Mann-Whitney U tests (Siegal, '56, pp. 116 cf) in which the litter is the experimental unit, litter percents for the two groups being contrasted are ranked for each character compared. This conservative non-parametric test was only performed on data for which t-tests yielded significant differences. Significance on the Mann-Whitney U test was defined at the $P < .05$ level († = $P < .05$).

Variants were said to show significant differences in compound-treated animals exclusive of any solvent or vehicle effects when they differed significantly from both VEH and UNTD. These are listed in the last three columns of the table for clarity.

Table taken from Beck, S.L. 1981. Teratology (in press)

Table 4. Significant differences in frequency of occurrence of each skeletal variant in each experiment when contrasted with untreated and vehicle-treated controls

	TFLT 1234	TCAP 1234	MBDM 1234	TB-I 12345	ACZM-I 123456	BUDR-I 123456	TB-II 12345	ACZM-II 123456	CORT 12345	DPH 12345	BUDR-II 123456789	Number P<.01 Compound vs Untd	Veh	Both
1. Short Snout	----	----	----	-----	------	------	-----	± v	-----	-----	--u------	0	0	0
2. Bent Nose	----	----	----	-u---	--u---	------	-----	------	-----	-----	--u------	-	-	-
3. Fused Nasals	----	----	----	-----	------	------	-----	±± vv	± v	u----	±	0	0	0
4. Nasal-Turbinal Fusion	----	----	----	-----	------	------	-----	------	-----	-----	---------	-	-	-
5. Nasal Fused to Skull	----	----	----	-----	-----u-	--u---	-----	--u---	-----	-----	---------	-	-	-
6. Preorbital Foramen Double	± V		±		± +± V	± v		++ v		± ±+ V	±± Vv	4	4	2
7. Preorbital Foramen Absent		u---	----	-----	---u--	------	-----	------	-----	-----	---------	-	-	-
8. Maxillary Foramen I Double	± v	±		± V			± V	± V	+ v			1	3	0
9. Maxillary Foramen I Absent	v	±		+ VV	± vv		± + v V	± v v	+ + VVvV	v	± +± +±	5	5	2
10. Maxillary Foramen II Double	+±±+ V		+			± v	+±+++ V	+ v	±+±++ v	V		9	1	0
11. Maxillary Foramen II Absent	+ ++ Vv		V V	± v v	±		±± v	+ ± Vv	± ± v	± vv v		2	2	0
12. Lachrymal-Maxillary Fusion	----	----	----	-----	------	------	-----	------	-----	-----	---------	-	-	-
13. Jugal Not Fused	----	----	----	-----	------	------	-----	------	-----	-----	---------	-	-	-
14. Interfrontal	+ v	+ + Vvv	± + vVV	++++ VVVV		+ + V V	++++ VVVV	++ V	+ v	± ±+ vV	+ ± + vV v V	18	15	13
15. I.F. - Frontal Fusion	± ± v v	+ V	v	V	±		+± +	± v		u----	v	3	2	1
16. Parted Frontals	±++ vVV	+++ V V	++ + V V	±±±± VvvV	±+ ± VV VV	++ V V v	++± + V	++±++ VVv V	+++ VVV	v	V	17	18	10
17. Fused Frontals	+ V		± v	± v		--u---	-----	±± vv	-----	----u	---------	1	1	1

Table 4. Continued.

	TFLT	TCAP	MBDM	TB-I	ACZM-I	BUDR-I	TB-II	ACZM-II	CORT	DPH	BUDR-II	Number P<.01 Compound vs		
	1234	1234	1234	12345	123456	123456	12345	123456	12345	12345	123456789	Untd	Veh	Both
18. Frontal Foramen Double	+ V	±	± v			±±	-u---	± v v		vvv	v vv	1	1	1
19. Frontal Foramen Absent			---u	u----		--u---	-----	-u----	-----	-----	---------	-	-	-
20. Accessory Parietal	+	+			++ VV	±+ vV	v	++ VV		VV	+ VV v	7	8	5
21. Foramen Palatinum Majus Double		± v	++++ V V		± +		±± ± vv	+		± + ± v v	V	5	1	0
22. Foramen Palatinum Anterior Double			± v	± v	+++ V		+ V	±				3	1	1
23. Foramen Palatinum Minus Anterior Absent	+ v				±±±± v		± v				± v	2	0	0
24. Foramen Palatinum Minus Posterior Double	+ V	----	----	-----	------	------	-----	------	-----	-----	---------	1	1	1
25. Foramen Palatinum Minus Posterior Absent	± V			± + v V	--u ---				-u---	-----	---------	1	2	1
26. Preoptic Sutures	+ ++ V v	v			± ± V V	±+±++ Vv v		±+ ± VV v	++ VV	++ VV	+ + + V V V	13	12	9
27. Abnormal Metoptic Roots	±		+ V	±+ v	++ vV	± + V	v	++ ± VV	± v	+ + VvvV		8	6	5
28. Pre-Basi Sphenoid Fusion	----	----	----	----u		------	-----	------	-----	-----	---------	-	-	-
29. Foramen Sphenoidale Mediale Present	v	± v		± v	±+± vV v						+± VVvVV	1	4	1
30. Posterior Border of the Palatine: Anteriad Suture	+ V				±	+ + VvVV v	±± ± v	+ V		-----	---------	2	3	2
31. Squamosal-Frontal Fusion	----	----	----	----	------	------	-----	------	-----	-----	---------	-	-	-

Table 4. Continued.

		TFLT 1234	TCAP 1234	MBDM 1234	TB-I 12345	ACZM-I 123456	BUDR-I 123456	TB-II 12345	ACZM-II 123456	CORT 12345	DPH 12345	BUDR-II 123456789	Number P<.01 Compound vs		
													Untd	Veh	Both
32.	Squasmosal-Parietal Fusion	----	----	----	-----	------	------	--u--	------	-u---		+ V	1	1	1
33.	Foramen Ovale Single	+ V	+ ±+ V	v	±+ + V v	± + vvV		± +	+ vV V	+ ± VvVVv	+ v		8	7	4
34.	Foramen Ovale Open Posteriorly	----	----	----	---u-	------	------	-----	------	-----	-----	---------	-	-	-
35.	Foramen Infra Ovale Double	± v V		± v	± v	± ± ± v v			± v	± ± v		+	1	1	0
36.	Foramen Infra Ovale Absent	± v	± ± v VV	v	+ VvvvV		±+±±±+ v v		v	v		± ± v v	2	3	0
37.	Foramen Pterygoideus Double	±	++ vv	±			± ++++ vv V	± v	±	±±		+ v	6	0	0
38.	Processus Pterygoideus Absent		± vv	----	-----	------	------	-----	------	-----	-----	---------	0	0	0
39.	Occipital-Periotic Fusion	----	---u	----	-----	------	------	-----	------	-----	-----	---------	-	-	-
40.	Interparietal-Occipital Fusion	----	----	----	-----	--u---	------	-u---	------	u----	-----	---------	-	-	-
41.	Basisphenoid-Basioccipital Fusion	----	----	----	-----	------	------	-----	------	-----	-----	---------	-	-	-
42.	Fenestra Flocculi Absent	±				++ V V	± ±	v	+ ± VVv v	± v	±	±+ V VV	2	5	1
43.	Foramina Hypoglossi Double			+ v	±± + VV V	v		± v Vv	v	+ v	+±	± ±± vv vv	4	4	1
44.	Mandibular Foramen Double	----	+ v	----	± V	±		± V	± v	+ ±± V		± v v	1	2	0
45.	Accessory Mental Foramen		+ V			±++ v v	±	± +± v	++ VV			±±± v v v	5.	2	2
46.	Dyssymphysis Atlas (C.1)		v	± v	+ V	------	± v	±++ vVV		±	-----	± V	3	4	3
47.	Foramina Transversaria Imperfecta Atlas (C.1)	±+ V	±		++++ VVVV	+ V	+±+++ V V v	++++ VVVV	± ± vvV		± + V	± +++±± + v vV V	16	12	11

Table 4. Continued.

		TFLT	TCAP	MBDM	TB-I	ACZM-I	BUDR-I	TB-II	ACZM-II	CORT	DPH	BUDR-II	Number P.< 01 Compound vs		
		1234	1234	1234	12345	123456	123456	12345	123456	12345	12345	123456789	Untd	Veh	Both
48.	Dyssymphysis Axis (C.2)	+ V	± v vV	± ± v v	++ Vv	± v	± vVv	+±+ V VV	+±+± vv	+ ± V	±	±+ ±+±± V v v	8	7	3
49.	Foramina Transversaria Imperfecta Axis (C.2)	±+ VV	+ V	V	++++ VVVV	+ VvV V	+ ±+++ Vv V	++±		± v		+ + + V V v	11	10	6
50.	Spinous Process C.3	± v	----	± v	+± V V	+ VVV	v	v	± v	±+± v v	±	+ ±++ + Vv V V	3	3	0
51.	Arch Foramen C.4 Absent	++ VVV		* u---	± v		-----u	----u	±± ± v v v	+ v		± ± v	3	3	2
52.	Arch Foramen C.5 Present	+ V		± + vvVV	± v	+ ±±++ V v V		±± ±	± ++ v Vv	+ VvVvV	+++ V	±±+ ++ ± v	10	6	3
53.	Foramina Transversaria Imperfecta C.3-C.6	+ + VV V	± V	+ V		± V	±±± v	V	± ±± v	±±±±		± V	2	7	1
54.	Accessory Transverse Foramina C.3-C.6	+ V	+± V		+±+ VVV	++ VVVvV		++++ VvV	+ ±++ v	± +±	+ + + V	± ± v	15	10	7
55.	Tubercula Anteriori C.6 Absent			± v		-----u	vv	-----	V	± ± vv v	v	+ ± ± v v	1	1	0
56.	Tubercula Anteriori C.6 Inflexed		* u---	v	±± ± v v		±+	± ± v		±± vv	vv	+ ±± VVV vv v	1	2	0
57.	Tubercula Anteriori C.6 Dystopic		----	-u--	± v	± v	± v	-----	--u---	-----			0	0	0
58.	Tubercula Anteriori C.6 Accessory	---u	----	----		-----u	--u---	-----	-----u	u----		---------	0	0	0
59.	Malformations (Dyss/Fus) C.3-C.7	± ± v v	± + v V	vv	+±±+ VvvV	± ± v V	+ ± V v	++++ vv	± + ±± v v v		±±± v vv	+ ± V vV	10	7	5
60.	Foramina Transversaria C.7		± v		± v vV	± vv	± v	± ± v			± vv V	±	0	2	0

Table 4. Continued.

	TFLT 1234	TCAP 1234	MBDM 1234	TB-I 12345	ACZM-I 123456	BUDR-I 123456	TB-II 12345	ACZM-II 123456	CORT 12345	DPH 12345	BUDR-II 123456789	Number P.<01 Compound vs Untd	Veh	Both
61. Malformations (Dyss/Fus) Th.1-Th.2		±±± v		± v	u-----	v v			±		± ±+ v vV	1	1	1
62. Spinous Process Th.2 Absent	± + vVVv	++	+ V		v	+ V v v		±		±±		4	3	2
63. Spinous Process Th.2 Reduced	+±++ V	± ± vv v	±		------	+ ± v	v	v	± ±	v		3	0	0
64. Spinous Process Th.2 Jointed	± ± vv		++ + V V		v		+ v V		± v v	± V	±± ++ vV v	5	4	2
65. Spinous Process Th.2 Dystopic	v	---u	+ ± v	----u	--u---	± v	-----	-u----	+	± vvvvv	±± v	2	0	0
66. Spinous Process Th.2 Accessory	± ± vvv	----		-----	------		-----	±± v v	-u---	-----	* u--------	0	0	0
67. Arch Foramina Thoracic Vertebrae	v		+		±	±+ v v	+ +++ VV v	+++++± V V v			+ ++ ++++ V V V	12	1	0
68. Reduced Articular Processes-Thoracic Vertebrae	± + v	± ± vvVV	+ V	+ v	+ ± V	+ V		+ + v V	+ +++ VV vv	± ± v v	± + V V	11	9	5
69. Malformations (Dyss/Fus) Th.3-Th.14	±± ± v	± +±		±	±± vvv	v	±±+++ v v	+	v	± v	± ± v	5	0	0
70. Fourteen Ribs	+ V	+ VVVV	V		++ ±+ VV VV	± + + vvV V	± vv	+++++ VVVVV	+ +	+ + VV VV	+ + V V v	15	20	12
71. Malformed Ribs	--u-	----	----	+ V	± ± v v	+ + V V	+ V	± v		-----	± + v V	5	5	5
72. Fused Sternebrae	V	v	± v	v	± v	± V	±+++ vVV	± ++	±	+ vV	++ V v	7	5	3
73. Malformed Sternebrae		±± VVv	++ V v	± ± vv V	±± + VV	±+ VV		++ VV	± ++± v		± ±+±±+ vVv	9	10	5

Table 4. Continued

		TFLT 1234	TCAP 1234	MBDM 1234	TB-I 12345	ACZM-I 123456	BUDR-I 123456	TB-II 12345	ACZM-II 123456	CORT 12345	DPH 12345	BUDR-II 123456789	Number P.<01 Compound vs Untd	Veh	Both
74.	Fossa Olecrani (Humerus) Perforata	+ v	+	+ V	±+ + v	± + v v	+ + V v	V	± + V	±+	V	± +± + ±+ v Vv V v	13	7	4
75.	Carpal Fusions	± v	± vv	±+++ v VV	+++ vVVV	+±+± V vvv	± v	+ +++ VVVVV	+ ±	v	± v	± ± ++ vV	12	9	7
76.	Sacralized L.6	-u--		----	± v	± v	± ± vv	u----	-----u	± v		---------	0	0	0
77.	Lumbar Fusions/Malf.	----	----	----	±± + v V	+ ++ V vV	± v		± + ±+ v V vV	-----	--u--	± +± + v VvvV	8	7	7
78.	27-Presacral Vertebrae	±± vv	+± V V	±	± ±±± vVVVV	±+ vV	+ + V V	++++ VVVV	++ ±± VV v	-----	±	+ ±+ + V vV V	12	17	12
79.	Sacral Fusions Dorsal Processes	± V		+ ± v		±+ ++ VV VV	±± v v	±++++ vvV V	+++++ vV v	-----		+ v	13	7	5
80.	Sacral Fusions Transverse Processes		-u--		± ±	+ ±+ V vV	------	V	+ v V	----u		+ V	4	5	4
81.	Sacral Fusions Vertebral Body		± vvv	± V v	+ V	++±++ vVvVV	± v	±+++ vVVV	+ V	-----	± v	± ±+ v V	10	10	9
82.	Caudalized S.3	++ V VV	+ V	± V v	v	± v	+ + V v	v	± ± V	± ++± v	+	V	7	7	2
83.	Foramina Acetabuli		± v	v		± ± v	u-----	----u	+ V V		-----	---------	1	2	1
84.	Dyssymphysis Ischio-Pubica			± vv	-----	------	--u---	* u----	v	-----	-----	---------	0	0	0
85.	Pubic Fusion	----	----	----	-----	------	------	-----	------	-----	-----	---------	-	-	-
86.	Tarsal Fusions	----	----	± v	± ±+ vvvV	± v	± ± v	±±+ vVVV		±±± v	-----	---------	2	4	2
87.	Fewer Than 30 Caudal Vertebrae	+++ Vv	+± Vv	++++ V V	±+ V	±+ V	V	±±±++ vVVVV	+ v	± v vV	v	+++ V	13	9	5
88.	Caudal Fusions			++ V	±±++ vvV	++ VV	v	+++ vVVV	++		± ±		10	6	6

Legend: Significance is based upon the results of t-tests performed on arcsin-square root transformed frequencies (Sokal and Rohlf 1969 (Biometry. W.H. Freeman, San Francisco, pp. 607-610), and is defined as P<.01 when discussed in the text. For the sake of completeness in this table, those traits in which P<.05 are also presented.

Abbreviations at the tops of the columns in each series are defined as follows:

TFLT: 2,4,5-T/Trifluralin experiments. 1. UNTD-VEH comparison, 2. 245T-L, 3. 245T-H, 4. Trifluralin
TCAP: Thalidomide/Captan experiments. 1. UNTD-VEH comparison, 2. THAL-L, 3. THAL-H, 4. Captan
MBDM: Maneb/Decamethrin. 1. UNTD-CMC Vehicle, 2. Maneb, 3. UNTD-Corn Oil Vehicle, 4. Decamethrin
TB-I, TB-II: Trypan Blue, series 1 and series 2. 1. UNTD-VEH comparison, 2. .037T, 3. .075T, 4. 0.15T, 5. 0.30T
ACZM-I, ACZM-II: Acetazolamide, series 1 and 2 respectively. 1. UNTD-VEH8 (note that UNTD in ACZM-II is the same group as UNTD in TB-II), 2. ACZM-L8, 3. ACZM-H8, 4. UNTD-VEH 9-11, 5. ACZM-L9-11, 6. ACZM-H9-11.
BUDR-I, BUDR-II: Bromodeoxyuridine, series 1 and 2. 1. UNTD-VEH 7, 2. BUDR-L7, 3. BUDR-H7, 4. UNTD-VEH 8, 5. BUDR-L8, 6. BUDR-H8. Groups 7-9 included in BUDR-II only; 7. UNTD-VEH 9, 8. BUDR-L9, 9. BUDR-H9
CORT: Cortisone 1. UNTD-VEH, 2. .15 CORT, 3. .3 CORT, 4. .6 CORT, 5. 1.2 CORT
DPH: Diphenylhydantoin. 1. UNTD-VEH, 2. 6.25 DPH, 3. 12.5 DPH, 4. 25 DPH, 5. 50 DPH.

See Table 2 for details of treatment and further explanation of treatment groups.

+ = P<.01 when contrasted with untreated

± = P<.05 when contrasted with untreated

V = P<.01 when contrasted with vehicle-treated group

v = P<.05 when contrasted with vehicle-treated group

u = unique observation. Trait seen in a single individual in this experimental series, in the group in which the letter appears.

* = unique observation in UNTD group. Other "u" designations in column 1 of an experimental series occurred in the vehicle-treated groups.

- = character not seen in this experimental series. Characters which differ from UNTD but not from VEH suggest solvent effects as do those which differ from VEH but not from UNTD. Characters which differ from both UNTD and VEH are likely due to the effects of the compound.

(Table 4 Legend)

Table 5. Number of significant (P<.01) and nearly significant (P<.05) differences from untreated and/or vehicle treated groups of skeletal variants in each experiment.

	No. of char. which vary	Number of variants which differ, compared with						
		UNTD		VEH		Both		
		<.01	<.05	<.01	<.05	<.01	U<.01,V<.05	U<.05,V<.01
TFLT	67							
1 Unt-Veh		6	6	6	6	6	0	0
2 245T-L		3	11	4	9	11	0	1
3 245T-H		19	8	16	8	11	3	2
4 Trifluralin		11	7	9	8	5	2	1
TCAP	61							
1 Untd-Veh		6	10	6	10	6	0	0
2 Thal-L		6	5	6	6	4	0	1
3 Thal-H		6	10	6	10	0	2	2
4 Captan		6	6	7	6	2	1	2
MBDM	62							
1 Untd-CMC		6	7	6	6	6	0	0
2 Maneb		10	6	4	9	0	2	1
3 Untd-Corn Oil		5	4	5	4	5	0	0
4 Decamethrin		12	7	12	9	8	0	0
TB-I	66							
1 Untd-Veh		2	6	2	6	2	0	0
2 .037T		6	10	7	8	4	0	2
3 .075T		6	10	8	7	6	0	2
4 0.15T		8	8	11	9	6	2	3
5 0.30T		18	12	24	10	14	2	4
TB-II	53							
1 Untd-Veh		5	9	5	9	4	0	0
2 .037T		10	13	8	10	4	2	3
3 .075T		16	7	11	5	8	1	2
4 0.15T		16	4	12	4	9	2	1
5 0.30T		22	7	17	9	15	3	0
ACZM-I	62							
1 Untd-Veh 8		3	4	3	4	3	0	0
2 ACZM-L8		4	9	5	5	2	2	2
3 ACZM-H8		12	10	13	7	12	0	0
4 Untd-Veh9-11		5	11	5	10	5	0	0
5 ACZM-L9-11		9	7	9	8	4	1	2
6 ACZM-H9-11		15	13	18	8	11	2	3

Table 5. Continued.

	No. of char. which vary	Number of variants which differ compared with UNTD		VEH		Both		
		<.01	<.05	<.01	<.05	<.01	U<.01,V<.05	U<.05,V<.01
ACZM-II	67							
1 Untd-Veh 8		3	6	3	6	3	0	0
2 ACZM-L8		10	7	8	10	5	1	1
3 ACZM-H8		16	12	12	14	9	5	0
4 Untd-Veh 9-11		3	6	3	5	3	0	0
5 ACZM-L9-11		12	7	5	4	4	0	0
6 ACZM-H9-11		19	11	15	12	11	3	1
BUDR-I	63							
1 Untd-Veh 7		5	6	5	6	5	0	0
2 BUDR-L7		3	7	1	9	0	0	0
3 BUDR-H7		13	9	13	9	9	0	0
4 Untd-Veh 8		4	6	4	6	4	0	0
5 BUDR-L8		5	9	3	5	1	0	2
6 BUDR-H8		12	6	5	12	5	3	0
BUDR-II	62							
1 Untd-Veh7		4	6	4	6	4	0	0
2 BUDR-L7		1	8	5	5	0	0	1
3 BUDR-H7		13	10	14	6	9	1	2
4 Untd-Veh8		4	5	4	7	4	0	0
5 BUDR-L8		6	8	4	8	1	1	1
6 BUDR-H8		14	9	12	8	9	1	1
7 Untd-Veh9		5	6	5	6	5	0	0
8 BUDR-L9		7	7	0	4	0	2	0
9 BUDR-H9		14	4	8	11	7	1	0
CORT	58							
1 Untd-Veh		6	8	6	8	6	0	0
2 0.15 CORT		4	8	2	9	0	2	0
3 0.3 CORT		6	12	3	5	1	1	1
4 0.6 CORT		8	7	4	5	2	1	0
5 1.25 CORT		8	8	4	9	2	2	1
DPH	62							
1 Untd-Veh		4	5	4	5	4	0	0
2 6.25 DPH		1	4	3	6	0	0	0
3 12.5 DPH		4	4	0	6	0	0	0
4 25 DPH		3	5	5	8	1	0	0
5 50 DPH		8	9	8	10	4	1	2

Table 5.

Legend:

TFLT = 2,4,5-T/Trifluralin experiments
TCAP = Thalidomide/Captan experiments
MBDM = Maneb/Decamethrin experiments
TB-I, TB-II = Trypan Blue experiments, series 1 and 2
ACZM-I, ACZM-II = Acetazolamide experiments, series 1 and 2
BUDR-I, BUDR-II = Bromodeoxyuridine experiments, series 1 and 2. Series 2 contains a group treated on day 9 of gestation
CORT = Cortisone experiments
DPH = Diphenylhydantoin experiments
UNTD = Untd = Untreated group
VEH = Veh = Vehicle treated group

Significance is based on the results of t-tests of arcsin-square root transformed frequencies of occurrence.

For additional details of treatment, refer to Table 2.

Table 6. Major differences between groups of untreated CD-1 mice as revealed by Chi-square analysis

Comparison	Experimental Series of Origin of Untreated Group						
	TFLT	TCAP	MBDM	TRBL-1	ACZM-1	BUDR	AZ/TB-2
All Groups	10,35,37, 44,51,52, 72,74,79, 82,83,87	22,26,37, 44,68,74, 87	21,37,44, 45,73, 75,82	37,44, 74,82	44	16,27, 37,53	70,73
TFLT vs CAPT (EPA)	26,35,45,49, 51,52,54,83						
Experiments at Wheaton College			37,45, 73	16,34	82	16,27,37, 53,74	35,70,82
Combined EPA vs Combined Wheaton College	16,22,25,35,37,44,51,52 68,72,74,79,82,83,87						
Uniquely variable in one group relative to all others	10,35,51, 52,72,79, 83	22,26,28	21,45 73,75	-	-	16,27,53	70,73
Highly variable from group to group	37,44,74,82						

Legend: I compared the frequencies of occurrence of each variant in each of the groups of untreated mice from the first seven groups of experiments. Comparisons included overall chi-square analyses for homogeneity and separate chi-square analyses for each variant, testing for homogeneity among the seven groups of untreated animals. By eliminating null class characters and characters in which the aggregate number in all seven groups was 25 or fewer (due to insensitivity of chi-square when expected is fewer than five individuals per cell), 41 individual characters remained to be tested. All but characters #20-Accessory Parietal, #23-Foramen Palatinum Minus Anterior Absent, #42-Fenestra Flocculi Absent, and #47-Foramina Transversaria Imperfecta of the Atlas, revealed significant heterogeneity (i.e. in each of 37 traits, $P<.01$, df=6, $x^2 > 16.8$). Numbers in the table are character numbers. Abbreviations are the same as those in Table 3. Characters were listed if they contributed a relative deviation (d^2/e) of > 10.0 to the Chi-square value in the comparison indicated. The first comparison tested a 41 by 7 contingency table.

Table 7. The number of characters which differ significantly between untreated groups of CD-1 mice.

	TFLT	CAPT	MBDM	TRBL-1	ACZM-1	BUDR	AZ-TB
TFLT	-	16	23	26	19	20	22
CAPT		-	23	18	16	17	19
MNBD			-	17	16	25	16
TRBL				-	10	15	14
ACZM					-	9	13
BUDR						-	13
AZ-TB							-

Legend: The number of variants among untreated CD-1 mice from each experimental series which, when compared by means of t-tests of arcsin square-root transformed frequencies with untreated from other series, compared two at a time, differ significantly (P<.01) in frequency of occurrence. (Example: untreated in TFLT series differ from untreated in the TCAP series of experiments with respect to frequencies of occurrence of 16 of the characters which show variation.) Abbreviations are the same as those used in Table 2, except that the second series of trypan blue and acetazolamide experiments were performed on the same "batch" of CD-1 mice and therefore shared the same untreated group. (abbr = AZ-TB)

Table 8. Two-Way Comparisons of Vehicle Effects.

Line Comparison

	Chi-Square Analyses	x^2	df[a]	P
1.	MBDM-Corn Oil vs MBDM-CMC	32.36	33	N.S.
2.	ACZM-1/$H_2$0-8 vs ACZM-1/$H_2$0-9x3	56.85	33	.01[b]
3.	BUDR-1/$H_2$0-7 vs BUDR-1/$H_2$0-8	20.32	31	N.S.
4.	ACZM-2/$H_2$0-8 vs ACZM-2/$H_2$0-9x3	43.69	32	N.S.

	Wilcoxon Matched-Pairs Signed Ranks[c]	T	N	z	P
5.	TRBL-1-VEH vs TRBL-2-VEH	750	61	-1.40	.0808
6.	ACZM-1/$H_2$0-8 vs ACZM-2/$H_2$0-9x3	973	62	-0.02	.4920
7.	ACZM-1/$H_2$0-9x3 vs ACZM-2/$H_2$0-9x3	1056	65	-0.11	.4562
8.	ACZM-1/$H_2$0-8 vs BUDR/$H_2$0-8	846	58	-0.07	.4721
9.	ACZM-2/$H_2$0-8 vs BUDR/$H_2$0-8	969.5	62	-0.05	.4801

Legend and Notes:

a. df = degrees of freedom; number of variants compared less one. Characters which would produce cells with an expected of less than 5 individuals were eliminated because of the restriction of the chi-square test.

b. One character, #54-Accessory Transverse Foramina C3-C6, contributed in a major way to the deviation from expectation. Eliminating that character results in a non-significant chi-square value in this comparison.

c. The Wilcoxon test is described in Seigal, S. (1956) Nonparametric Statistics; McGraw-Hill, N.Y.

Systematic effects of a particular solvent, if present, should occur each time that solvent is used to challenge animals; interactions between solvent and "batch" of animals would be revealed by different results using the same Vehicle treatment in different lots of mice; handling effects would be suggested if different Vehicles applied to the same lot of mice resulted in similar patterns of response. That the solvent effects are partly handling effects is suggested by the fact that different solvents presented to the same group of animals (line 1) produce homogeneous results, and two different solvent regimes in the same group of mice (lines 3, 4) also have very similar consequences. Systematic effects of a particular solvent are more difficult to ascertain because the frequencies among untreated vary so greatly. By considering the differences between each untreated and vehicle-treated group for each variant, then comparing those differences with one another when the same vehicle is applied to different "batches" of mice using a non-parametric test of significance (lines 5-9), systematic vehicle effects may be suggested by the similarity of identical vehicle treatments applied at different times. However, vehicle effects are in general slight, and homogeneity between experiments may simply be a reflection of random variation in both series.

Table 9. Summary of Significant Differences ($P<.01$) for Sensitive or Important Skeletal Variants.

Character Number	Character Name	No. of Expt. Series		Untd vs Veh	Compound vs			Terat Dose vs		
		Untd	Veh		Untd	Veh	Both	Untd	Veh	Both
14.	Interfrontal	10	8	2	18	15	13	10	8	7
16.	Parted Frontals	9	10	4	17	18	10	10	12	7
20.	Accessory Parietal	6	5	1	7	8	5	5	4	3
26.	Preoptic Sutures	6	7	1	13	12	9	10	9	7
27.	Abnormal Metoptic Roots	6	5	1	8	6	5	4	3	3
33.	Foramen Ovale Single	8	6	2	8	7	4	7	4	3
47.	Foramina Transversaria Imperfecta Atlas (C1)	7	8	4	16	12	11	7	6	5
48.	Dyssymphysis Axis (C2)	6	7	2	8	7	3	8	6	5
49.	Foramina Transversaria Imperfecta Axis (C2)	7	7	5	11	10	6	4	4	3
54.	Accessory Transverse Foramina C3-C6	8	6	2	15	10	7	6	5	4
59.	Malformations (Dyss/Fus) C3-C7	6	5	0	10	7	5	6	6	4
68.	Reduced Articular Processes Thoracic Vertebrae	8	7	1	11	9	5	8	8	5
70.	Fourteen Ribs	8	8	3	15	20	12	10	13	8
71.	Malformed Ribs	4	4	0	5	5	5	5	5	5
73.	Malformed Sternebrae	6	7	1	9	10	5	5	5	4
74.	Fossa Olecrani Perforata (Humerus)	9	6	0	13	7	4	10	4	4
75.	Carpal Fusions	6	5	3	12	9	7	6	4	4
77.	Lumbar Fusions/ Malformations	4	4	0	8	7	7	7	7	7
78.	27 Presacral Vertebrae	6	7	1	12	17	12	8	10	8
79.	Sacral Fusions - Dorsal Processes	5	4	1	13	7	5	6	4	3
80.	Sacral Fusions Transverse Processes	3	4	0	4	5	4	4	5	4
81.	Sacral Fusions Veretbral Body	5	6	0	10	10	9	6	7	6
87.	Fewer than 30 Caudal Vertebrae	8	8	3	13	9	5	9	4	3
88.	Caudal Fusions	5	4	1	10	6	6	5	3	3
	(Maximum number possible)	(11)	(11)	(17)	(42)	(42)	(42)	(19)	(19)	(19)

Table 9.

Legend:

The table was extracted from the data in Table 4, and indicates the number of times the character presented was significantly different from untreated (Untd) or vehicle-treated (Veh) controls, or both. The table may be interpreted as follows: There were eleven different Experimental Series, and #14-Interfrontal, for example, in one or more groups within the series, had a frequency which was significantly different from Untd in 10 of the eleven and from Veh in 8 of the 11 series. In all there were 59 comparisons possible for contrast with Untd or with Veh. Of these 17 were Untd-Veh comparisons and 42 were comparisons of compound-treated groups with Untd or Veh. Thus Interfrontal differed in 18 of 42 compound treated groups so compared. This variant differed in frequency from 15 of the 42 compound-Veh comparisons, and in 13 of the 15 cases the frequency was different from Untd as well as Veh, arguing for strong compound effects on this character in at least 13 experimental groups. (It should be mentioned that only 37 comparisons could be made for #77, 54 comparisons for #78,79 and 44 comparisons for #80, since there were one or more series in which these variants did not occur at all.) There were, in the 11 experimental series, 19 assorted teratogenic doses of various compounds administered. In 10 of the 19 teratogenic dose groups the frequency of occurrence of Interfrontal was significantly different from Untd and in 8 teratogenic-dose groups Interfrontal differed from Veh. In 7 of the 8 Vehicle differences, the frequency was also different from Untd. Thus Interfrontal is both a sensitive indicator for many compounds at teratogenic doses, and also an important indicator for a subtantial number of compounds to which a fetus was exposed at lower, non-teratogenic doses.

Table 10. Variants which responded in a major way in both series of trypan blue experiments.

	0.3 TRBL				0.15 TRBL				.075 TRBL			
	UNTD		VEH		UNTD		VEH		UNTD		VEH	
	I	II	I	II	I	II	I	II	I	II	I	II
14 Interfrontal	*	*	*	*	*	*	*	*	*	*	*	*
46 Dyss. Atlas (C.1)	*	*	*	*		*		*		o		o
47 F.T.I. Atlas (C.1)	*	*	*	*	*	*	*	*	*	*	*	*
48 Dyss. Axis (C.2)	*		o	*	*		*	*		*		
49 F.T.I. Axis (C.2)	*	o	*		*	*	*		*	*	*	
54 Acc. Trans. for C.3-C.6	*	*	*	*	o	*	*	o	*	*	*	*
59 Dyss/Fus C.3-C.7	*	*	*	o	o	*	o	o	o	*	o	
71 Malformed Ribs	*	*	*	*								
75 Carpal Fusions	*	*	*	*	*	*	*	*	*	*	*	*
78 27-Presacral Vert.	o	*	*	*	o	*	*	*	o	*	*	*
81 Sacral Fusions-Vert.	*	*	*	*		*		*		*		*
86 Tarsal Fusions	*	*	*	*	o	*	o	*		o	o	*
87 <30 Caudal Vert.	*	*	*	*	o	*		*		o		*
88 Caudal Fusions	*	*	*	*	*	*	o	*	o	*	o	*

Legend: 0.3 TRBL = 0.3% trypan blue treatment; 0.15 TRBL and .075 TRBL are the .15% and .075% treatments, respectively. Numbers with variant names correspond with the 88 character list.

I = Series I; II = Series II
UNTD = Comparison with Untreated
VEH = Comparison with Vehicle
* = Significant difference (P<.01) in frequency of experimental, based on t-test; o = P<.05, included for completeness.

Table 11. Variants which responded in a major way in both series of Acetazolamide experiments

	ACZM-L8				ACZM-H8				ACZM-L9-11				ACZM-H9-11			
	UNTD		VEH		UNTD		VEH		UNTD		VEH		UNTD		VEH	
	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	I
16 Parted Frontals		*	*	*	*	*	*	*		*		*		*	*	*
20 Accessory Parietal	*	*	*	*	*	*	*	*								
26 Preoptic Sutures				*		*	*	*							*	
27 Abnormal Metoptic Roots	*	*	o	*	*	*	*	*								
33 Foramen Ovale Single								*					*	*	*	*
45 Accessory Mental Foramen									*	*		*	*	*	o	*
47 F.T.I. Atlas (C.1)													*		*	*
52 Arch Foramen C.5									*	*		*	*	*	*	
54 Accessory Transverse Foramina C.3-C.6		*	*		*		*							*	*	
68 Red. Artic Proc - Thor. Vert.					*	*	*							*		*
70 Fourteen Ribs	*	*	*	*	*	*	*	*		*	*	*	*	*	*	*
73 Fused Sternebrae										*	*	*	*	*	*	*
74 Fossa Olecrani Perforata													*	*	o	*
77 Lumbar Fusions/Malf					*	*	*	*	*	o			*	*	*	*
78 27-Presacral Vertebrae		*		*	*	*	*	*								
79 Sacral Fusions - Dorsal Processes		*	*		*	*	*		*	*	*		*	*	*	
80 Sacral Fusions - Transverse Proc.					*		*						*	*	*	*
81 Sacral Fusions - Vertebral Body	*				*		*		*		*		*	*	*	*
83 Foramina Acetabuli															*	*
87 Fewer Than 30 Caudal Vertebrae													*	*	*	*
88 Caudal Fusions									*	*	*	*	*	*	*	*

Legend:

ACZM - L8, H8, L9-3, H9-3 represent low and high doses on day 8 of gestation and on days 9-11 respectively. Numbers alongside variant names correspond with the 88 character list.

I = Series I; II = Series II; UNTD = Comparison with Untreated; VEH = Comparison with Vehicle-treated group.

* = Significant difference (P<.01) in frequency of the experimental from the comparison group, based on t-test; o = P<.05 based on a t-test (not considered significant, but included for completeness).

Table 12. Variants which responded in a major way in both series of bromodeoxyuridine experiments.

	BUDR-H7				BUDR-L7				BUDR-H8				BUDR-L8			
	UNTD		VEH		UNTD		VEH		UNTD		VEH		UNTD		VEH	
	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II
14 Interfrontal	*	*	*	*	*		*		*	o	*	o				
20 Accessory Parietal	*		*	*			o					o				
26 Preoptic Sutures	*	*	*	*	*				*	*	o	*				
48 Dyssymphysis Atlas		*	*	*		o				*				o		
59 Malf. (Dyss/Fus) C.3-C.7	*	*	*	*		o				o	o	*				o
68 Red. Artic. Proc. Thoracic Vert.	*	*	*	*						*		*			o	
70 Fourteen Ribs	*	*	*	*			o		*	*	*	*				
71 Malformed Ribs	*	o	*	o					*	*	*	*				
73 Malformed Sternebrae						o			*	*	*	*		o	*	o
74 Fossa Olecrani Perforata	*	*		*			*		*	*	o	*				
78 27-Presacral Vertebrae	*	*	*	*					*	*	*	*				
82 Caudalized S3	*		*	*					*		*					

Legend:

BUDR-H7,L7,H8,L8 represent high and low doses on day 7 and on day 8 of gestation, respectively.

I = Series one; II = Series two; UNTD = comparison with untreated; VEH = comparison with appropriate vehicle-treated group.

* = Significant difference (P<.01) in frequency of the experimental from the comparison group, based on t-tests; o = P<.05, based on t-test (not considered significant, but included for completeness)

Table 13. Frequencies of occurrence (%) of skeletal variants among a group of CD-1 mice at 62±2 days post natal and significant differences in frequencies among experimental groups in a second series of experiments involving prenatal exposure to bromodeoxyuridine, contrasted with untreated and vehicle-treated groups.

		UNTD	Veh 7	Low 7	High 7	Veh 8	Low 8	High 8	Veh 9	Low 9	High 9
	N =	97	99	97	87	96	98	100	93	96	91
1.	Short Snout	-									
2.	Bent Nose	-									
3.	Fused Nasals	0						a			
4.	Nasal-Turbinal Fusion	-									
5.	Nasal Fused to Skull	-									
6.	Preorbital Foramen Double	33.0					Va	a,b			
7.	Preorbital Foramen Absent	-									
8.	Maxillary Foramen I Double	66.0									
9.	Maxillary Foramen I Absent	0		a			5.1	a		5.2	a
10.	Maxillary Foramen II Double	14.4									
11.	Maxillary Foramen II Absent	1.0									
12.	Lachrymal-Maxillary Fusion	-									
13.	Jugal Not Fused	-									
14.	Interfrontal	9.3		b	34.5V			a,b			28.3V
15.	I.F.-Frontal Fusion	1.0		b							
16.	Parted Frontals	30.9									V
17.	Fused Frontals	-									
18.	Frontal Foramen Double	1.0		b			b	b			
19.	Frontal Foramen Absent	-									
20.	Accessory Parietal	4.1	0	V	V			b			
21.	Foramen Palatinum Majus Double	22.7									
22.	Foramen Palatinum Minus Anterior Double	55.7									
23.	Foramen Palatinum Minus Anterior Absent	2.1		a,b							
24.	Foramen Palatinum Minus Posterior Double	-									
25.	Foramen Palatinum Minus Posterior Absent	-									
26.	Preoptic Sutures	24.7			50.6V			66.0V			68.5V
27.	Abnormal Metoptic Roots	33.0									
28.	Pre-Basisphenoid Fusion	-									
29.	Foramen Sphenoidale Mediale Present	45.4		V	69.0V	a	V	V			

Table 13 Continued

N =	UNTD 97	Veh 7 99	Low 7 97	High 7 87	Veh 8 96	Low 8 98	High 8 100	Veh 9 93	Low 9 96	High 9 91
30. Palatine Posterior Border: Anteriad Suture	-									
31. Squamosal-Frontal Fusion	-									
32. Squamosal-Parietal Fusion	0					5.1V				
33. Foramen Ovale Single	28.9									
34. Foramen Ovale Open Posteriorly	-									
35. Foramen Infra Ovale Double	7.2						21.0			
36. Foramen Infra Ovale Absent	0									a,b
37. Processus Pterygoideus Double	7.2						0			
38. Processus Pterygoideus Absent	-									
39. Occipital-Periotic Fusion	-									
40. Interparietal-Occipital Fusion	-									
41. Basisphenoid-Basioccipital Fusion	-									
42. Fenestra Flocculi Absent	5.2		a	26.4		V	V			
43. Foramina Hypoglossi Double	64.9	a	b		a				a,b	a,b
44. Mandibular Foramen Double	72.2					b				b
45. Accessory Mental Foramen	25.8				a	a		a		b
46. Dyssymphysis Atlas (C.1)	1.0			V						
47. Foramina Transversaria Imperfecta Atlas (C.1)	35.1	a		11.5	10.4	16.3	a	15.1		15.2
48. Dyssymphysis Axis (C.2)	0		a	12.6V		a	4.0	a	a	b
49. Foramina Transversaria Imperfecta Axis (C.2)	17.5		5.2		5.2			5.4		b
50. Spinous Process C.3	9.3	1.0	b		0	0	b		1.0	
51. Arch Foramen C.4 Absent	2.1									
52. Arch Foramen C.5 Present	74.2	a	a	43.7		54.1	54.0		a	
53. Foramina Transversaria Imperfecta C.3-C.6	6.2		a	V						
54. Accessory Transverse Foramina C.3-C.6	18.6	a								a
55. Tubercula Anteriori C.6 Absent	0			4.6		a		a		b
56. Tubercula Anteriori C.6 Inflexed	0	4.0	V	V			a,b	a		b

Table 13. Continued

N =	UNTD 97	Veh 7 99	Low 7 97	High 7 87	Veh 8 96	Low 8 98	High 8 100	Veh 9 93	Low 9 96	High 9 91
57. Tubercula Anteriori C.6 Dystopic	0									
58. Tubercula Anteriori C.6 Accessory	-									
59. Malformations (Dyss/Fus) C.3-C.7	1.0			10.3V		b	Va			
60. Foramina Transversaria C.7	44.3						a			
61. Malformations (Dyss/Fus) Th.1-Th.2	0					a,b	4.0V			
62. Spinous Process Th.2 Absent	8.2									
63. Spinous Process Th.2 Reduced	1.0									
64. Spinous Process Th.2 Jointed	16.5	a	V						2.1b	4.3
65. Spinous Process Th.2 Dystopic	0		a		a					
66. Spinous Process Th.2 Accessory	-									
67. Arch Foramina Thoracic Vertebrae	97.9	88.9		82.8	88.5		87.0	79.6	76.0	87.0
68. Reduced Articular Processes-Thoracic Vertebrae	3.1			V			14.0V			
69. Malformations (Dyss/Fus) Th.3-Th.14	1.0						a			
70. Fourteen Ribs	53.6			90.8V			93.0V			b
71. Malformed Ribs	0						13.0V			
72. Fused Sternebrae	16.5							35.5	37.5	b
73. Malformed Sternebrae	43.3		a			a,b	13.0V	a	a	20.7
74. Fossa Olecrani (Humerus) Perforata	27.8	a		4.6V	a		4.0V		a	7.6b
75. Carpal Fusions	3.1						a		17.7b	31.5V
76. Sacralized L.6	-									
77. Lumbar Fusions/Malf.	0						4.0V	a	b	27.2V
78. 27-Presacral Vertebrae	0			10.3V		a,b	5.0V			16.3V

Table 13. Continued

N =	UNTD 97	Veh 7 99	Low 7 97	High 7 87	Veh 8 96	Low 8 98	High 8 100	Veh 9 93	Low 9 96	High 9 91
79. Sacral Fusions Dorsal Processes	5.2					0b				
80. Sacral Fusions Transverse Processes	1.0									13.0^{V}
81. Sacral Fusions Vertebral Body	3.1					a,b			a	16.3V
82. Caudalized S.3	7.2		V							
83. Foramina Acetabuli	-									
84. Dyssymphysis Ischio-Pubica	-									
85. Pubic Fusion	-									
86. Tarsal Fusion	-									
87. Fewer Than 30 Caudal Vertebrae	4.1							0.0	0.0	0.0
88. Caudal Fusions	4.1									
No. of Significant Differences										
Compared with UNTD	-	4	1	13	4	6	14	5	7	13
Compared with VEH	4	-	5	14	-	4	12	-	0	8
Average Difference (%)										
Significant Variants vs UNTD	-	4.4	-12.4	6.0	-13.9	-7.8	3.1	-7.1	-1.1	5.4
vs VEH	4.4	-	10.7	14.3	-	-0.2	8.1	-	0	22.9
Absolute Value vs UNTD	-	6.4	12.4	20.2	13.9	10.0	16.3	14.7	12.8	19.2
vs VEH	6.4	-	11.7	16.6	-	14.2	16.5	-	0	22.9
All Variants vs UNTD	-	-1.9	-0.1	2.1	-1.8	-2.1	0.2	-0.8	-0.5	2.0
vs VEH	-1.9	-	1.8	3.9	-	-0.3	2.0	-	0.3	2.9
Absolute Value vs UNTD	-	3.8	3.5	7.1	3.9	4.3	6.8	4.7	4.5	6.6
vs VEH	3.8	-	3.9	6.8	-	3.7	6.3	-	3.3	6.6
No. EXPTL > Control										
vs UNTD	-	18	26	38	27	19	30	22	27	32
vs VEH	-	-	37	41	-	24	34	-	27	33
No. EXPTL < Control										
vs UNTD	-	37	24	20	27	38	29	32	27	23
vs VEH	-	-	18	19	-	29	26	-	23	21

Legend for Table 13.

Treatments are described in Table 2. Values in the experimental columns indicate significant differences from UNTD ($P<.01$) based on t-test comparisons of arcsin-square root transformed frequencies.

V = Significant differences from the vehicle-treated group
a = Frequency $P<.05$ when compared with UNTD
b = Frequency $P<.05$ when compared with VEH group
N = Number of specimens in each group, presented at the top of each column
- = Character did not vary in this experimental series.

The number of variants for which the experimental frequency was higher than control frequency (EXPTL > Control) plus the number in which experimental frequency was lower than control frequency (EXPTL < Control) do not necessarily add up to the number of variants since in some cases values were unaffected by treatment and were identical in the two groups being compared. C = Cervical, Th = Thoracic, L = Lumbar, S = Sacral vertebrae; Dyss = Dyssymphysis, Fus = Fusions, Malf- Malformations.

Table 14. Differences in frequency of occurrence of skeletal variants among four-way outcross (CBHA=C57BL/Gr, CBA/Gr, C3H/HeH, A/Gr) mice subjected to selection for differences in response to trypan blue teratogenesis.

					Gen 3						Gen 6 UNTD			Gen 7						Gen 9						
	CD-1/G1	CD-1/G2	G1/G2	G2T/G2U	S/U	R/U	C/U	S/C	R/C	S/R	S/U	R/U	S/R	S/U	R/U	C/U	S/C	R/C	S/R	S1/S2	R1/R2	S1/R1	S2/R2	S/C	R/C	S/R
1. Short Snout	-	-	-	T																						
2. Bent Nose				T										S	R					1						
3. Fused Nasals	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4. Nasal-Turbinal Fusion	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5. Nasal Fused to Skull	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6. Preorbital Foramen Double	G	G																								
7. Preorbital Foramen Absent	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8. Maxillary Foramen I Double																C				1		S				
9. Maxillary Foramen I Absent															R			R	R							
10. Maxillary Foramen II Double	D	D	1																	1						
11. Maxillary Foramen II Absent													S							-	-	-	-	-	-	-
12. Lachrymal-Maxillary Fusion	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13. Jugal Not Fused	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14. Interfrontal				T	S	R	C	S	R					S	R	C		R					R		R	
15. I.F.-Frontal Fusion											-	-	-	S			S					S				S
16. Parted Frontals				T	S	R	C			S	S															
17. Fused Frontals	-	-	-	-	-	-	-	-	-	-					V					-	-	-	-	-	-	-
18. Frontal Foramen Double																										
19. Frontal Foramen Absent	-																									
20. Accessory Parietal			-											-	-	-	-	-	-							
21. Foramen Palatinum Majus Double								C	C						V			C								
22. Foramen Palatinum Minus Anterior Double							U	S												1			R		R	
23. Foramen Palatinum Minus Anterior Absent	D	D	-	-	-	-	-	-	-	-	-	-	-					C		-	-	-	-	-	-	-
24. Foramen Palatinum Minus Posterior Double	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25. Foramen Palatinum Minus Posterior Absent				T											R											
26. Preoptic Sutures	G	G			S									U		U										
27. Abnormal Metoptic Roots	D			T							U						C							C		
28. Pre-Basisphenoid Fusion	-	-	-	-	-	-	-	-	-	-	-	-	-		R			C								
29. Foramen Sphenoidale Mediale Present	G																							C		

Table 14. Continued

					Gen 3						Gen 6 UNTD			Gen 7						Gen 9						
	CD-1/G1	CD-1/G2	G1/G2	G2T/G2U	S/U	R/U	C/U	S/C	R/C	S/R	S/U	R/U	S/R	S/U	R/U	C/U	S/C	R/C	S/R	S1/S2	R1/R2	S1/R1	S2/R2	S/C	R/C	S/R
30. Palatine Posterior Border: Anteriad Suture	G	G				R			R	R		U	R			U				1			R			R
31. Squamosal-Frontal Fusion	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
32. Squamosal-Parietal Fusion	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-							
33. Foramen Ovale Single	G			U	U	U		C	C			U		U	U	U		C	S							
34. Foramen Ovale Open Posteriorly	-	-	-	-																						
35. Foramen Infra Ovale Double														S	R								R			
36. Foramen Infra Ovale Absent														S		C				2						
37. Processus Pterygoideus Double	G	G			U			C												1						
38. Processus Pterygoideus Absent	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
39. Occipital-Periotic Fusion	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
40. Interparietal-Occipital Fusion	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
41. Basisphenoid-Basioccipital Fusion	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
42. Fenestra Flocculi Absent												U	S		U				S							
43. Foramina Hypoglossi Double	D	D									U				R		C		R			R				R
44. Mandibular Foramen Double	D	D	1								S			S	R					1			R			
45. Accessory Mental Foramen	D	D												U	U			C						C	C	
46. Dyssymphysis Atlas (C.1)	-	-	-	T																						
47. Foramina Transversaria Imperfecta Atlas (C.1)			-		-	-	-	-	-	-	-	-	-													
48. Dyssymphysis Axis (C.2)																						R				R
49. Foramina Transversaria Imperfecta Axis (C.2)			-	T																						
50. Spinous Process C.3					-	-	-	-	-	-	-	-	-				S	R		1						
51. Arch Foramen C.4 Absent	G	G		T		R	C													2				S	R	
52. Arch Foramen C.5 Present	D	D																		1			R			
53. Foramina Transversaria Imperfecta C.3-C.6															R			R	R						R	
54. Accessory Transverse Foramina C.3-C.6		D				R																		C		
55. Tubercula Anteriori C.6 Absent	-	-	-																	-	-	-	-	-	-	-
56. Tubercula Anteriori C.6 Inflexed			-	-	-	-	-	-	-	-				U	U	U										

Table 14. Continued

					Gen 3						Gen 6 UNTD			Gen 7						Gen 9						
	CD-1/G1	CD-1/G2	G1/G2	G2T/G2U	S/U	R/U	C/U	S/C	R/C	S/R	S/U	R/U	S/R	S/U	R/U	C/U	S/C	R/C	S/R	S1/S2	R1/R2	S1/R1	S2/R2	S/C	R/C	S/R
57. Tubercula Anteriori C.6 Dystopic	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
58. Tubercula Anteriori C.6 Accessory	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-							
59. Malformations (Dyss/Fus) C.3-C.7																										
60. Foramina Transversaria C.7	D	D																								
61. Malformations (Dyss/Fus) Th.1-Th.2			-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
62. Spinous Process Th.2 Absent			-	T							S		S													
63. Spinous Process Th.2 Reduced				T	S						S		S	S	R											
64. Spinous Process Th.2 Jointed	-	-	-	-										-	-	-	-	-	-							
65. Spinous Process Th.2 Dystopic	-	-	-	-	-	-	-	-	-	-				-	-	-	-	-	-	-	-	-	-	-	-	-
66. Spinous Process Th.2 Accessory	G	G		U	U	U							R	U		U				2	2					
67. Arch Foramina Thoracic Vertebrae	D	D																		1						
68. Reduced Articular Processes-Thoracic Vertebrae		G	2									U							S							
69. Malformations (Dyss/Fus) Th.3-Th.14			-	-	-	-	-	-	-	-	-	-	-													
70. Fourteen Ribs	D	D									-	-	-						R							
71. Malformed Ribs	-				-	-	-	-	-	-	-	-	-													
72. Fused Sternebrae								V	C				R					R	R							
73. Malformed Sternebrae																				1			R	C		R
74. Fossa Olecrani (Humerus) Perforata					U																1					
75. Carpal Fusions	D	D		T	S	R	C	S	R			R	R	S	R	C		R	R	1			R		R	R
76. Sacralized L.6	G	G		U	U			U				R	R	U	R	U		R	R			R	R		R	R
77. Lumbar Fusions/Malf.		G			-	-	-	-	-	-	-	-	-						R		2		R			
78. 27-Presacral Vertebrae		-									-	-	-							-	-	-	-	-	-	-

Table 14. Continued

					Gen 3						Gen 6 UNTD			Gen 7						Gen 9						
	CD-1/G1	CD-1/G2	G1/G2	G2T/G2U	S/U	R/U	C/U	S/U	R/C	S/R	S/U	R/U	S/R	S/U	R/U	C/U	S/C	R/C	S/R	S1/S2	R1/R2	S1/R1	S2/R2	S/C	R/C	S/R
79. Sacral Fusions Dorsal Processes	G	G										U										R	R	C		R
80. Sacral Fusions Transverse Processes				T	-	-	-	-	-	-	-	-	-		R				R			R	R	C		R
81. Sacral Fusions Vertebral Body	G	G										U							R			R		C		R
82. Caudalized S.3	G	G										R	R	U			C		R	1			R			R
83. Foramina Acetabuli					U			U						S	R											
84. Dyssymphysis Ischio-Pubica	-	G									-	-	-							-	-	-	-	-	-	-
85. Pubic Fusion	-	-	-	-	-	-	-	-	-	-	-	-	-							-	-	-	-	-	-	-
86. Tarsal Fusion	-			T	S	R	C				S	R		S	R	C		R	R	1		R	R		R	R
87. Fewer Than 30 Caudal Vertebrae	G										S	R			R		S	R								
88. Caudal Fusions				T	S						-	-	-	S		C						S		S		S
No. of Differences P<.01	24	24	3	18	13	12	6	9	6	3	8	11	10	17	22	12	6	13	16	17	3	10	14	10	7	13
No. of Variant Characters	60	63	57	65	59						56			66						66						

Legend: Trypan blue treatment consisted of i.p. injection of 0.25cc of 0.3% trypan blue in 0.9% NaCl on days 7,8, and 9 of gestation.

S = susceptible line - affected litters were used for establishing each subsequent generation.

R = resistant line - all-normal litters thrown by treated dams were used to establish each subsequent generation.

U = unselected line - maintained by matings of animals whose dams were not treated.

C = subsamples of unselected line which were treated to provide base level frequencies for each generation.

See Beck '71, J.E.Z. 177 for details of experimental protocols.

Entries in the table indicate significant differences in the comparison described at the top of each Column. Letter or number indicates which of the two groups compared had the higher frequency of occurrence.

Columns 1 and 2 - Comparison between the untreated foundation generations of CBHA outcross G1 = F1 hybrids of C57Bl x CBA and C3H x A crosses; G2 = Fourway outcross from (C57/CBA)F1 x (C3H/A)F1. These are the foundation population of CBHA four-way outcross lines; CD-1 = untreated mice from the Maneb/Decamethrin experimental series. G = CBHA; D = CD-1

Table 14. Continued.

Legend:

Columns 3 and 4 - Comparisons between untreated foundation generation animals (G1 vs G2) and between treated (G2T) and untreated (G2U) G2 mice. 1 = G1; 2 = G2; T = progeny of treated G2 dams; U = progeny of untreated G2 dams

Columns 5-10 - Gen 3 - the third generation CBHA mice. Note that S and R were established from G2 litters whose dams were treated and U from G2 litters whose dams were untreated.

Columns 11-13 - Gen 6 - the sixth CBHA generation. Selection was relaxed (i.e. none of the dams of any of these animals was treated) following three generations (G3-5) of selection.

Columns 14-19 - Gen 7 - the seventh CBHA generation. Resumption of selection: S, R, and C were from dams which had been treated during gestation; U were from unselected mice whose dams were not treated.

Columns 20-26 - Gen 9 - Ninth CBHA generation. Two separate rounds of treatment (1 and 2) were performed. In some cases the same dam contributed litters to both rounds. Note a substantial number of differences between S1 and S2, with S1 generally having the higher frequencies. R1 and R2 agreed very closely with one another.

Figures 1-95 Photographic Atlas of Skeletal Variants

Legend:

1. Short Snout
2. Bent Nose
3. Fused Nasals
4. Nasal-Turbinal Fusion
5. Nasal Fused to Skull
6. Preorbital Foramen Double
7. Preorbital Foramen Absent
8. Maxillary Foramen I Double
9. Maxillary Foramen I Absent
10. Maxillary Foramen II Double
11. Maxillary Foramen II Absent
12. Lachrymal-Maxillary Fusion
13. Jugal Not Fused
14. Interfrontal
15. I.F.-Frontal Fusion
16. Parted Frontals
17. Fused Frontals
18. Frontal Foramen Double
19. Frontal Foramen Absent
20. Accessory Parietal
21. Foramen Palatinum Majus Double
22. Foramen Palatinum Minus Anterior Double
23. Foramen Palatinum Minus Anterior Absent
24. Foramen Palatinum Minus Posterior Double
25. Foramen Palatinum Minus Posterior Double
26. Preoptic Sutures
27. Abnormal Metoptic Roots
28. Pre-Basisphenoid Fusion
29. Foramen Sphenoidale Mediale Present
30. Palatine Posterior Border: Anteriad Suture
31. Squamosal-Frontal Fusion
32. Squamosal-Parietal Fusion
33. Foramen Ovale Single
34. Foramen Ovale Open Posteriorly
35. Foramen Infra Ovale Double
36. Foramen Infra Ovale Absent
37. Processus Pterygoideus Double
38. Processus Pterygoideus Absent
39. Occipital-Periotic Fusion
40. Interparietal-Occipital Fusion
41. Basisphenoid-Basioccipital Fusion
42. Fenestra Flocculi Absent
43. Foramina Hypoglossi Double
44. Mandibular Foramen Double
45. Accessory Mental Double
46. Dyssymphysis Atlas (C.1)
47. Foramina Transversaria Imperfecta Atlas (C.1)
48. Dyssymphysis Axis (C.2)
49. Foramina Transversaria Imperfecta Axis (C.2)
50. Spinous Process C.3
51. Arch Foramen C.4 Absent
52. Arch Foramen C.5 Present
53. Foramina Transversaria Imperfecta C.3-C.6
54. Accessory Transverse Foramina C.3-C.6
55. Tubercula Anteriori C.6 Absent
56. Tubercula Anteriori C.6 Inflexed
57. Tubercula Anteriori C.6 Dystopic
58. Tubercula Anteriori C.6 Accessory
59. Malformations (Dyss/Fus) C.3-C.7
60. Foramina Transversaria C.7
61. Malformations (Dyss/Fus) Th.1-Th.2
62. Spinous Process Th.2 Absent
63. Spinous Process Th.2 Reduced
64. Spinous Process Th.2 Jointed
65. Spinous Process Th.2 Dystopic
66. Spinous Process Th.2 Accessory
67. Arch Foramina Thoracic Vertebrae
68. Reduced Articular Processes-Thoracic Vertebrae
69. Malformations (Dyss/Fus) Th.3-Th.14

70. Fourteen Ribs
71. Malformed Ribs
72. Fused Sternebrae
73. Malformed Sternebrae
74. Fossa Olecrani (Humerus) Perforata
75. Carpal Fusions
76. Sacralized L.6
77. Lumbar Fusions/Malf.
78. 27-Presacral Vertebrae
79. Sacral Fusions Dorsal Processes
80. Sacral Fusions Transverse Processes
81. Sacral Fusion Vertebral Body
82. Caudalized S.3
83. Foramina Acetabuli
84. Dyssymphysis Ischio-Pubica
85. Pubic Fusion
86. Tarsal Fusion
87. Fewer Than 30 Caudal Vertebrae
88. Caudal Fusions

squam = squamosal bone
par = parietal bone
c = cervical vertebra
th = thoracic vertebra

car = carpal bone
cen = centrale
tar = tarsal bone

The numbers used to represent the variants correspond to those presented in Table 4, and used consistently throughout this report. The letter "A" after a variant number indicates the absence of that variant or the alternate mode of expression. In a few instances, this was used as sufficient to locate the variant for the reader; in other cases both forms are presented and labeled; in some cases specimens with unilateral expression of a bilateral trait were photographed and the alternate mode of expression, while present, was not labeled. One variant is unrepresented (#87) because of its obvious nature and difficulty in photographing it. Several are closely related to variants which are indicated (#7, 9, 57, 58, 81), and were infrequent or difficult to photograph. Thus the reader can examine the related variants (6A, 8, 55, 56, 79, 80) and infer the unphotographed variants. Several variants never occurred (#4, 5, 12, 13, 34, 37, 41, 85), but their locations, if they were present, could be inferred from related variants.

1
3A

2
1,2

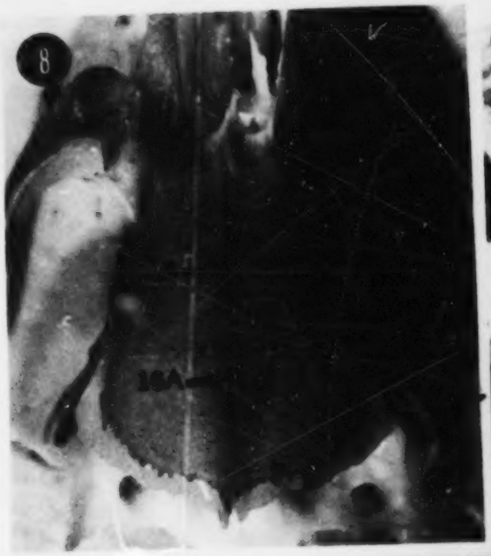
3
2
1A

3
4

5
6A
18A
19A

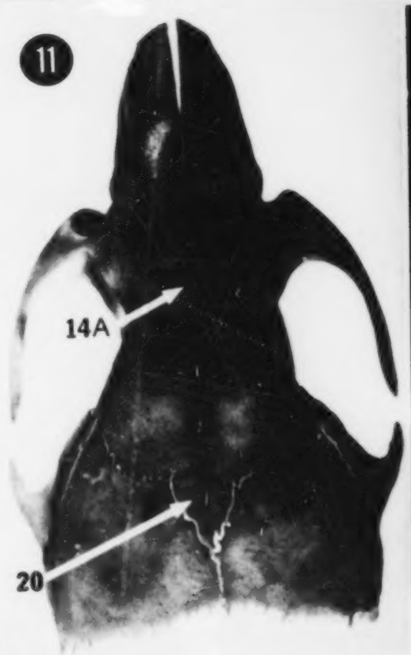
7
10

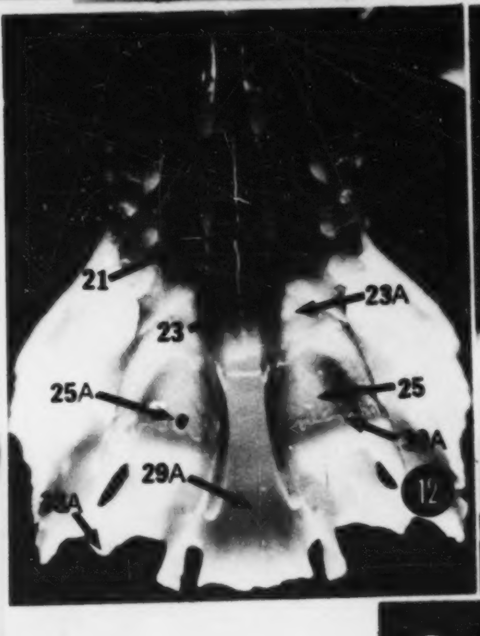
6
6

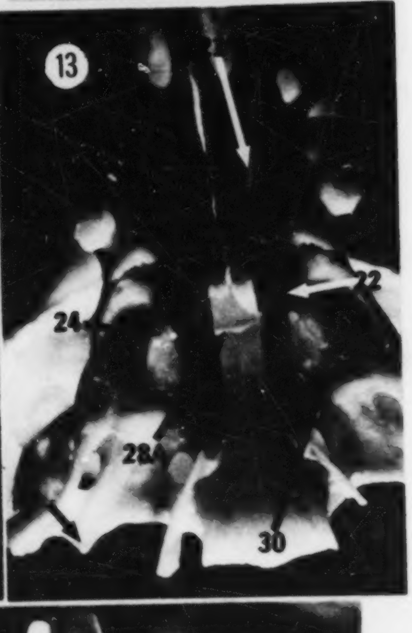

8
16A

9
17

10


11
14A
20


21
23A
23
25A
25
29A
12


13
22
24
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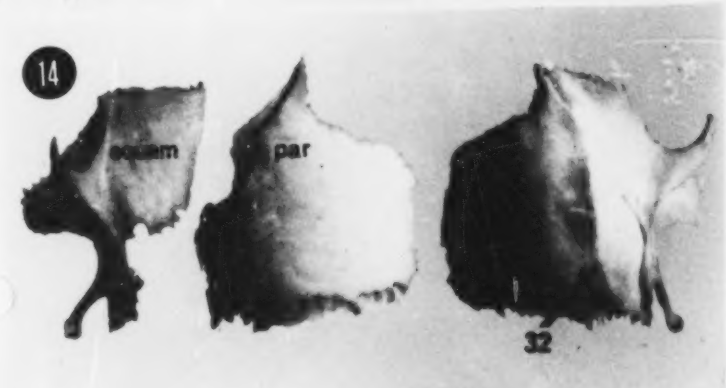
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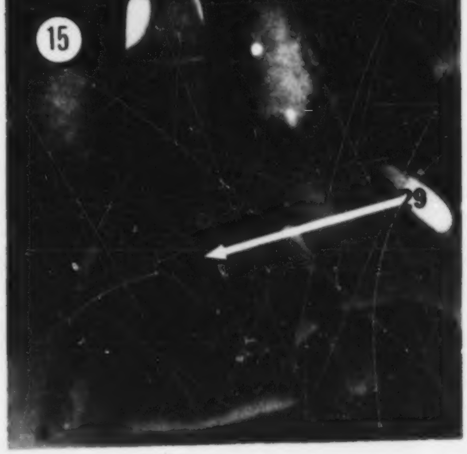
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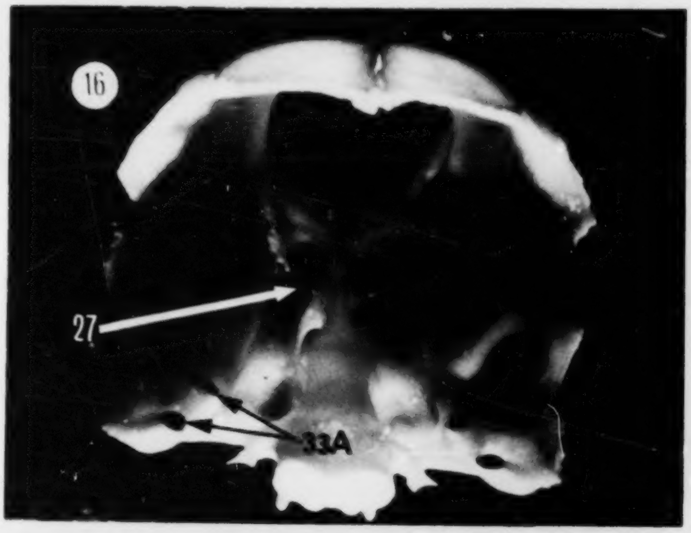
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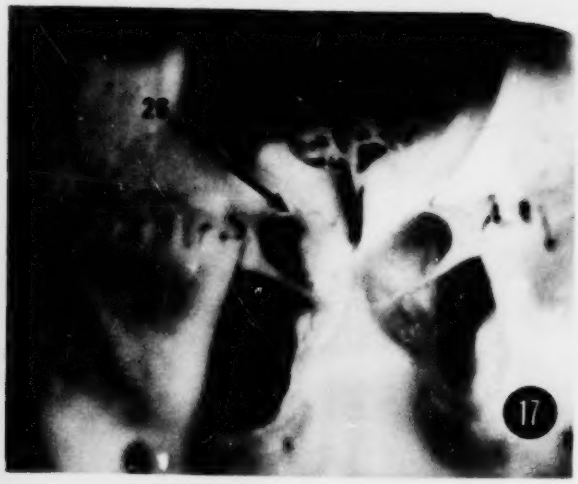
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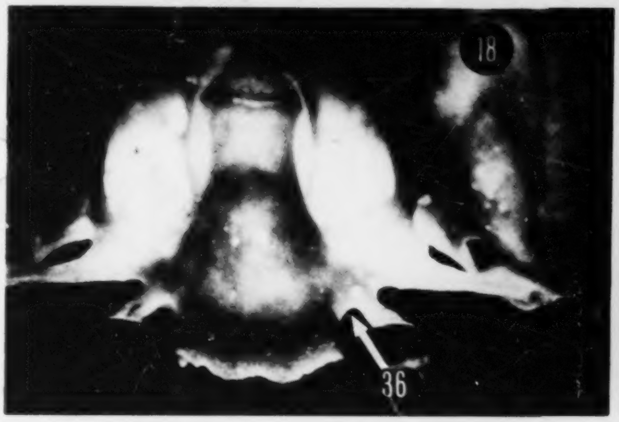
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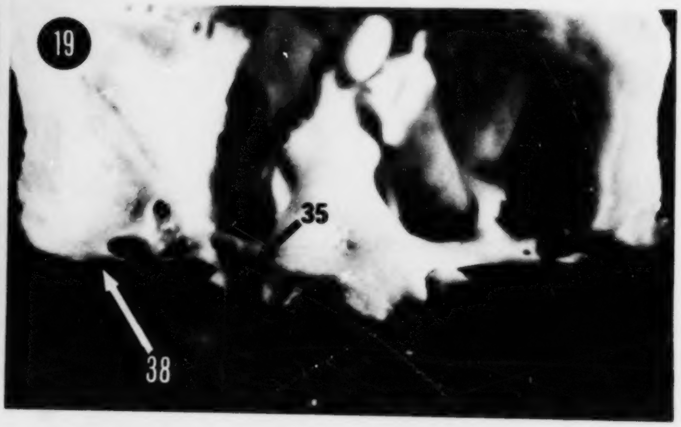
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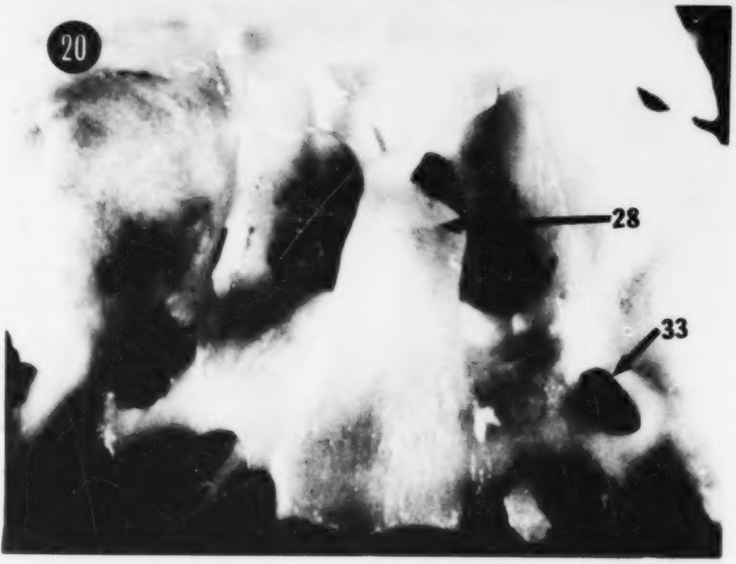
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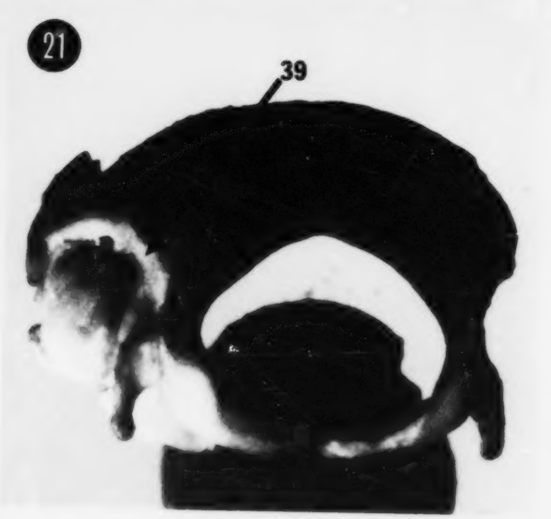
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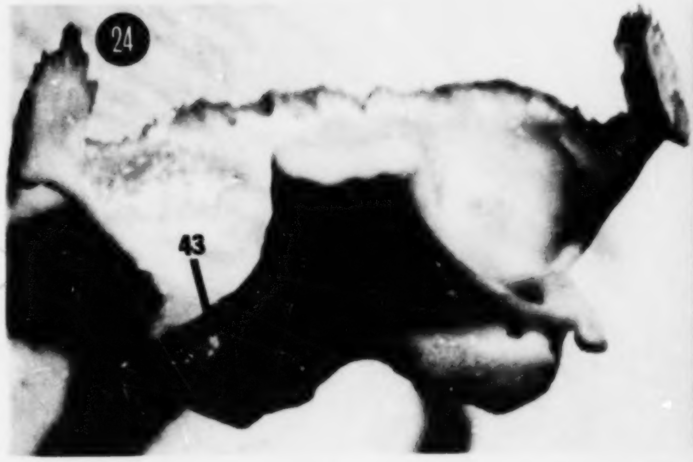
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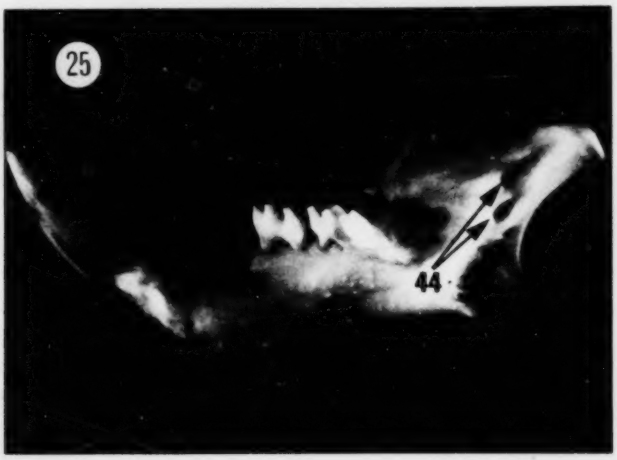
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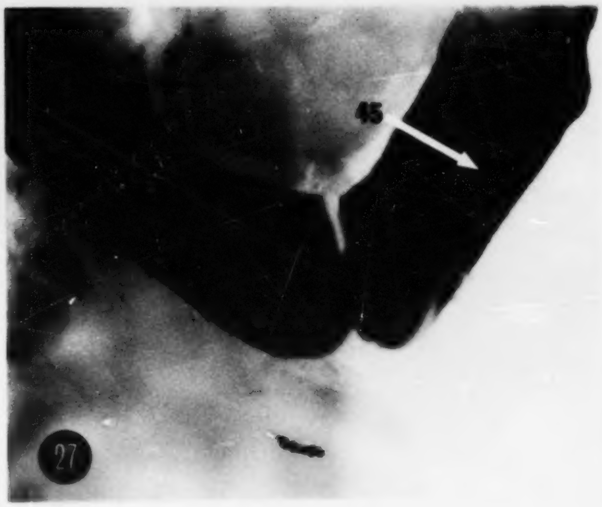
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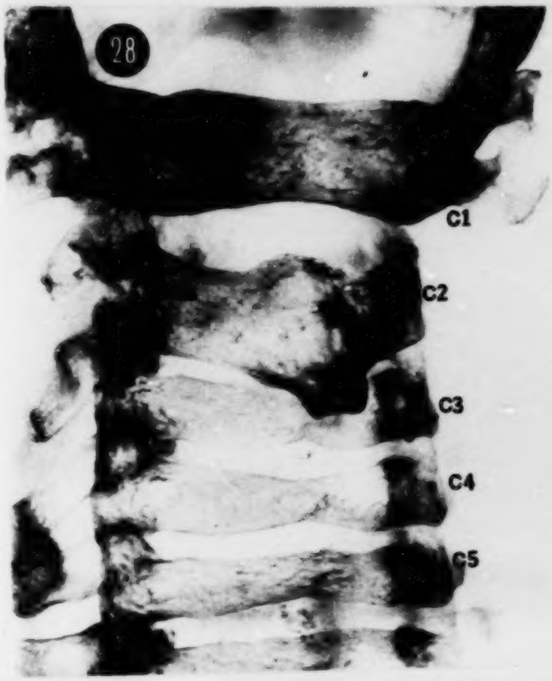
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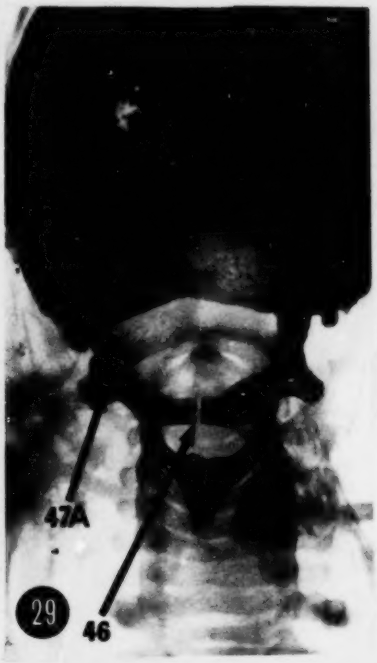
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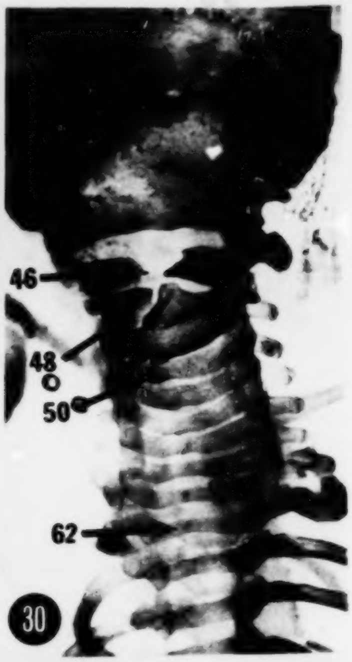
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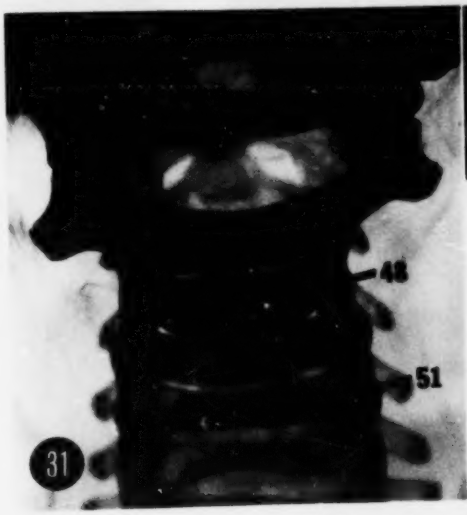
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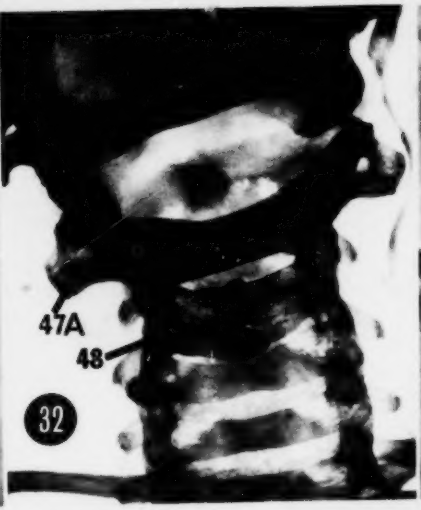
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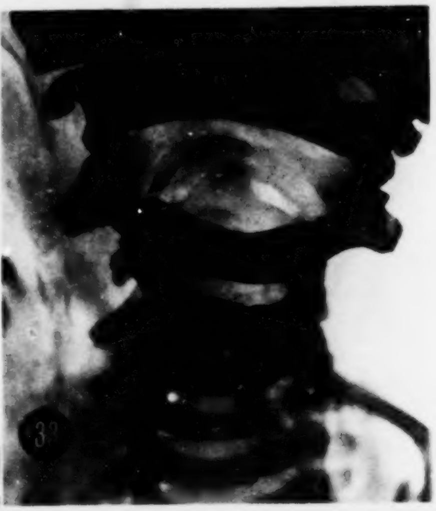
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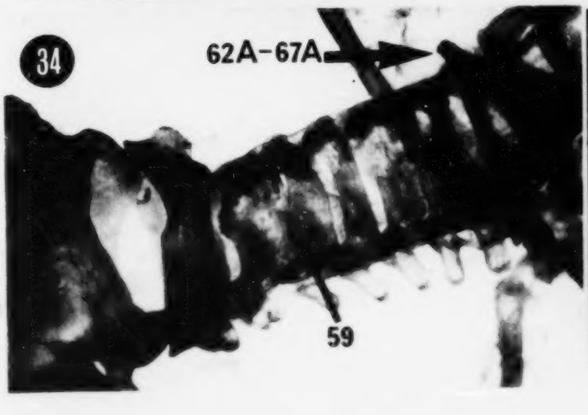
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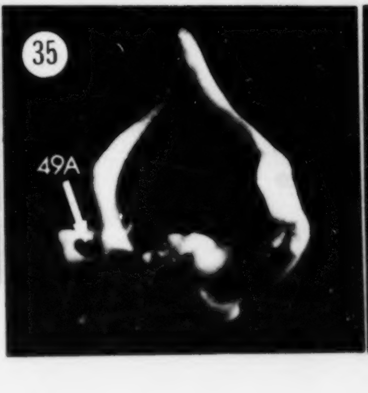
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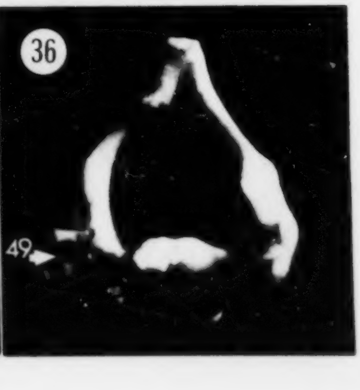
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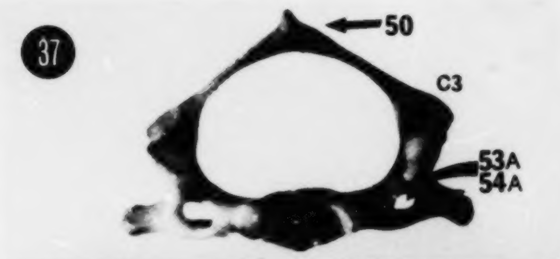
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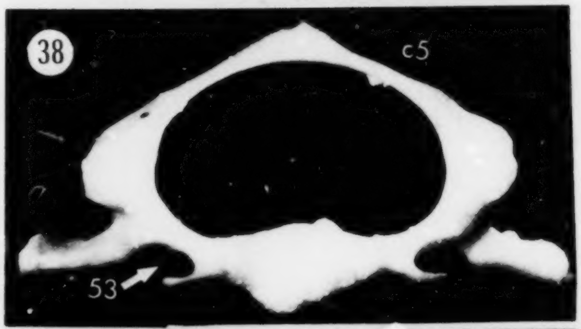

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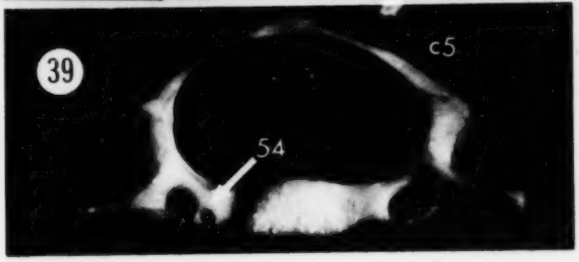

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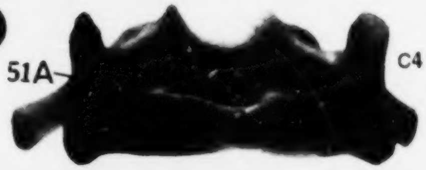

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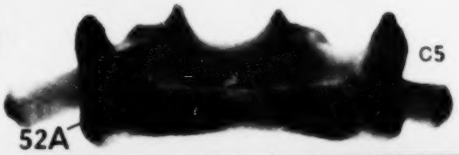

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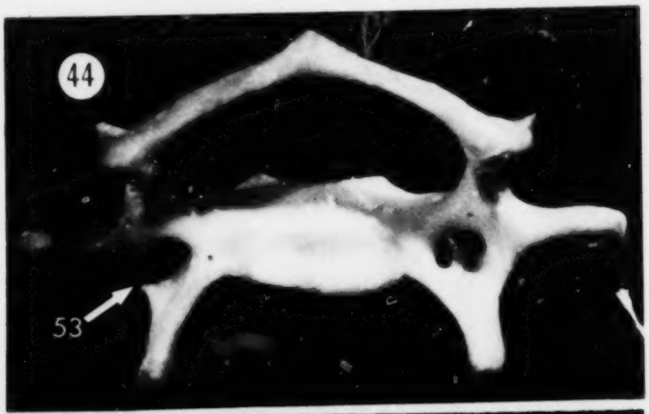

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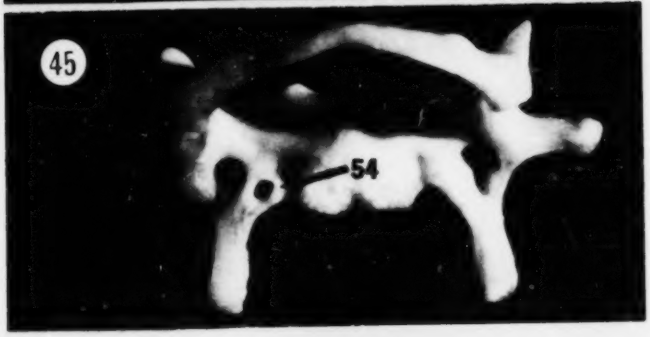

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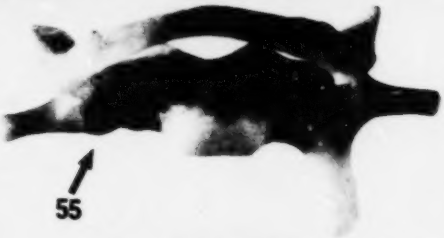

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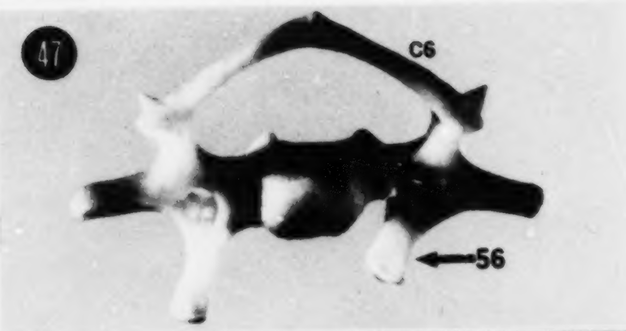

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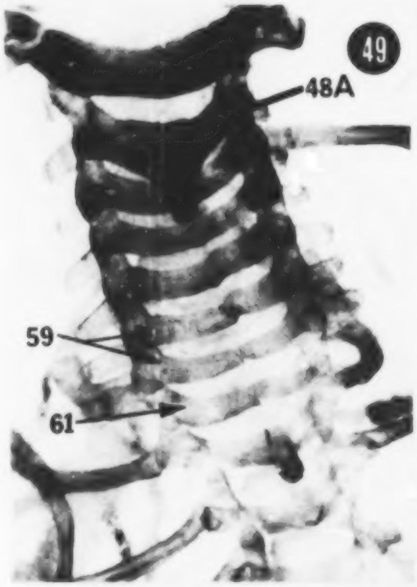
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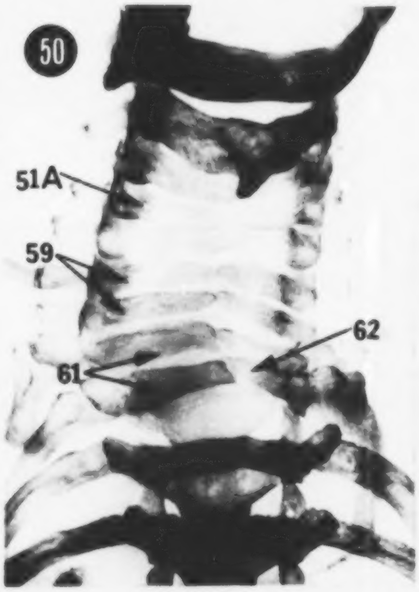
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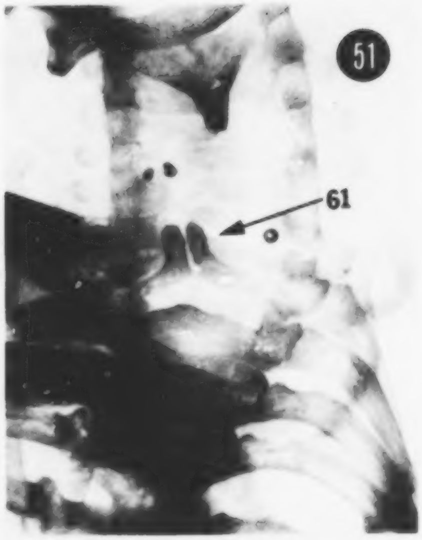
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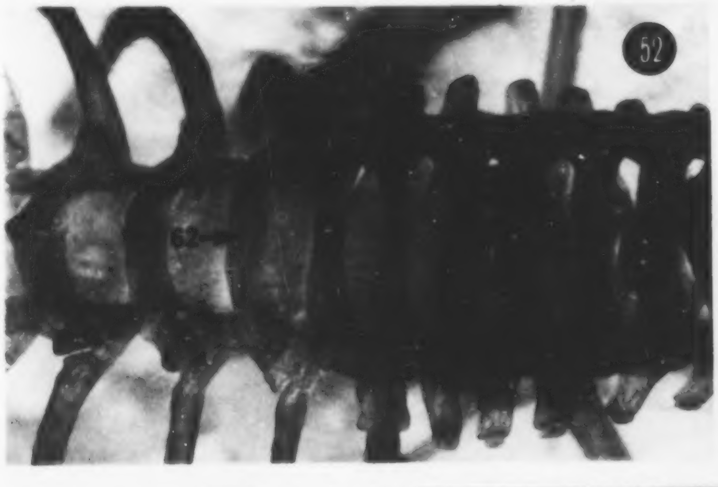
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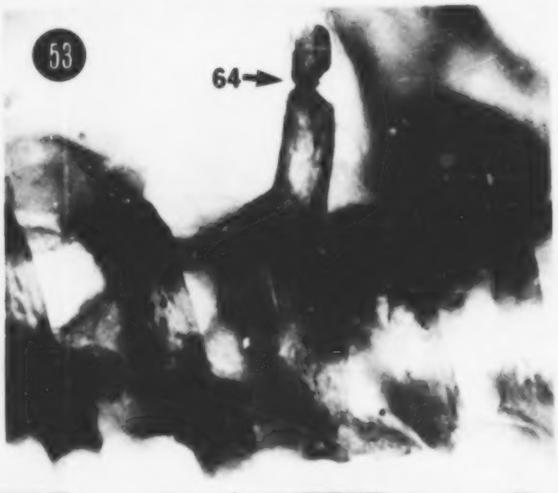
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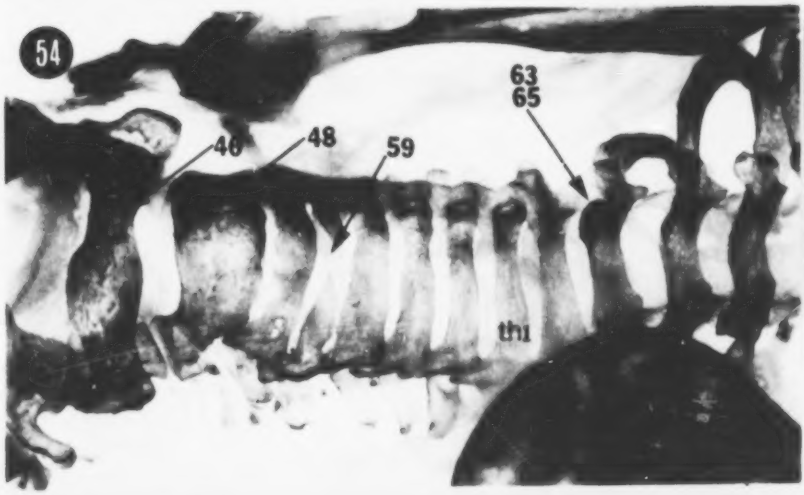
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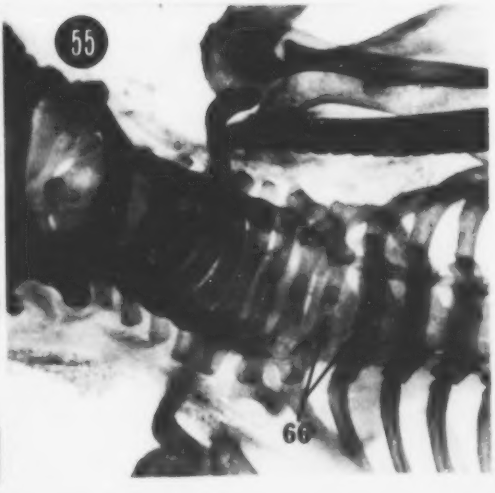
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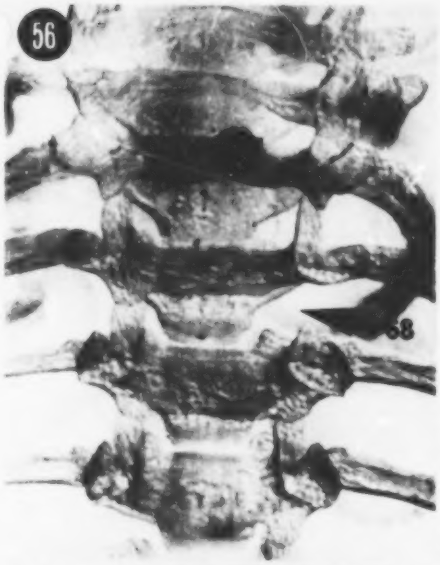
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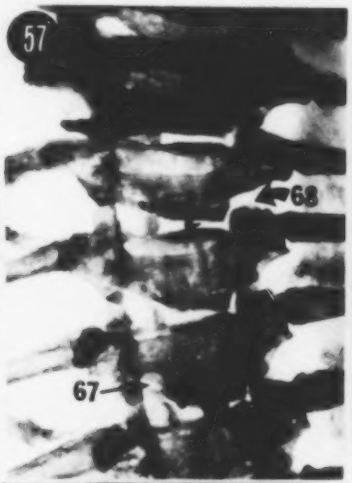
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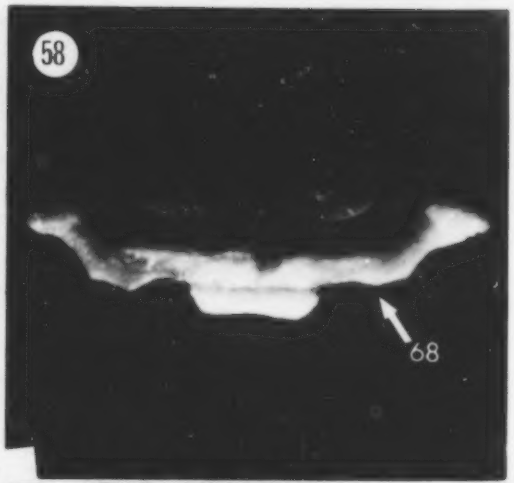
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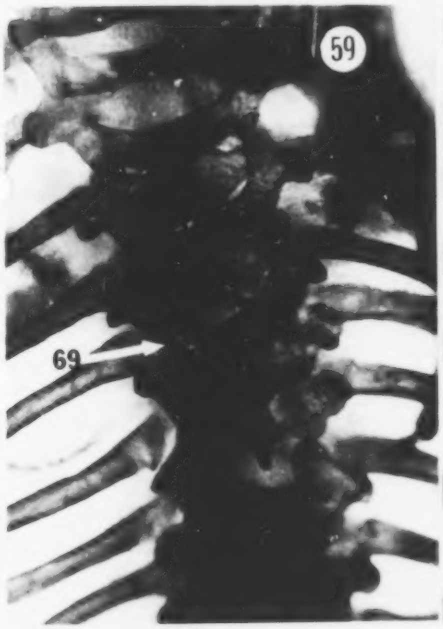
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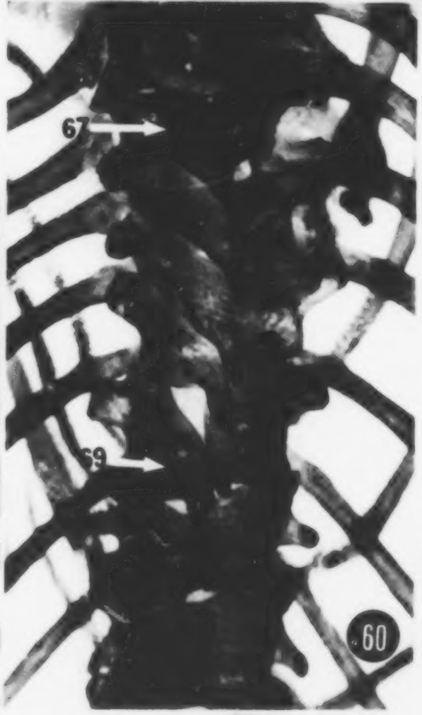
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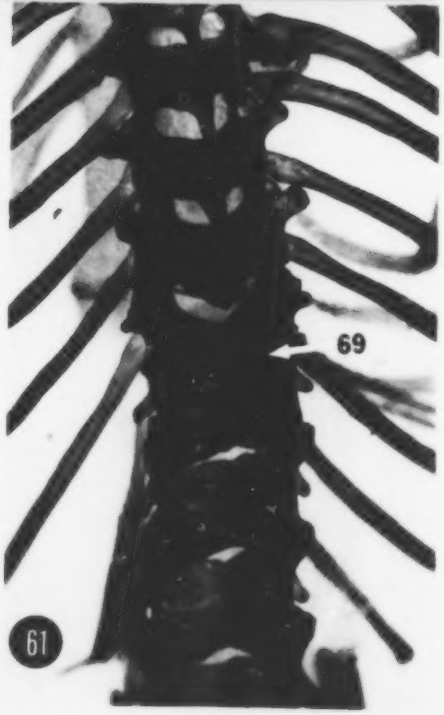
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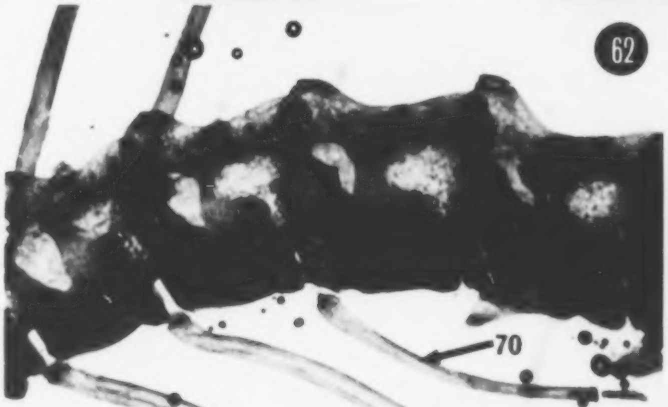
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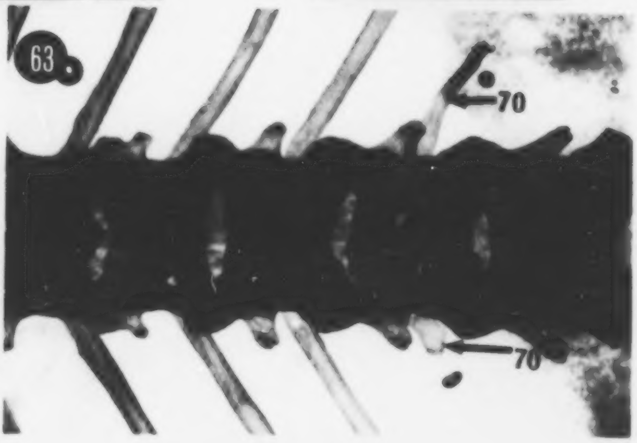
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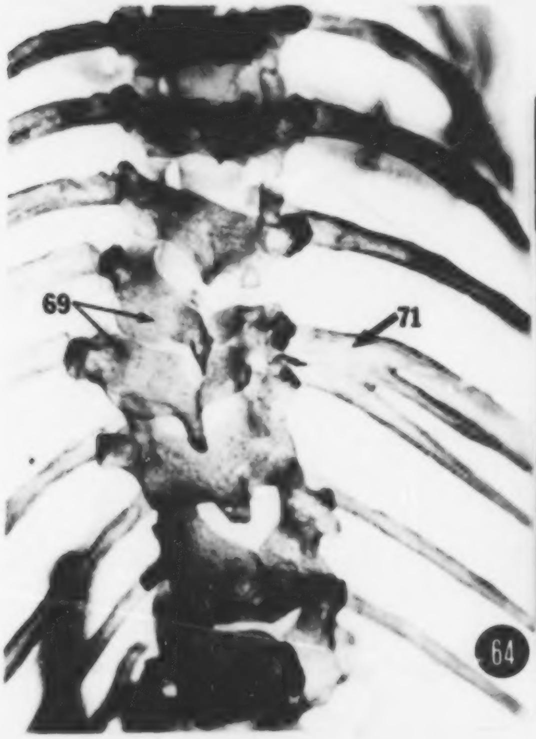

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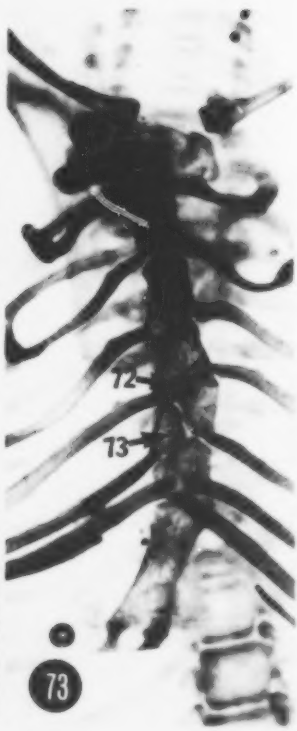

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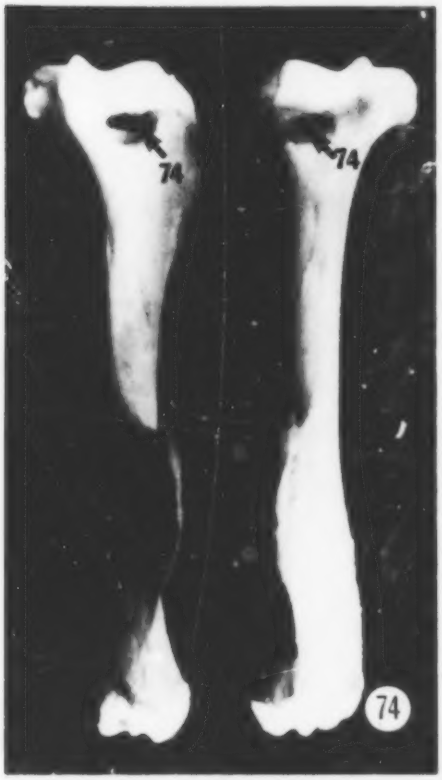

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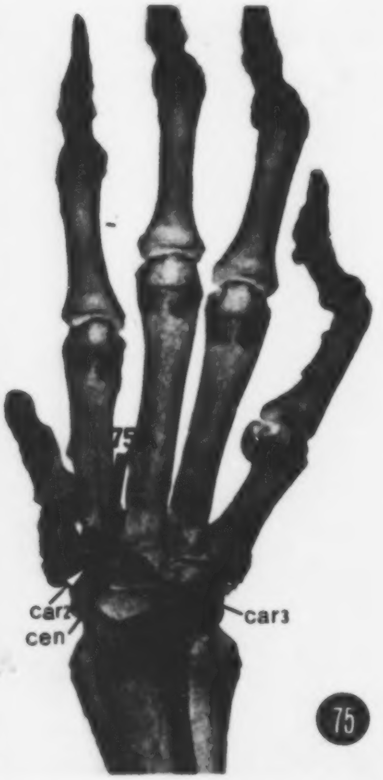
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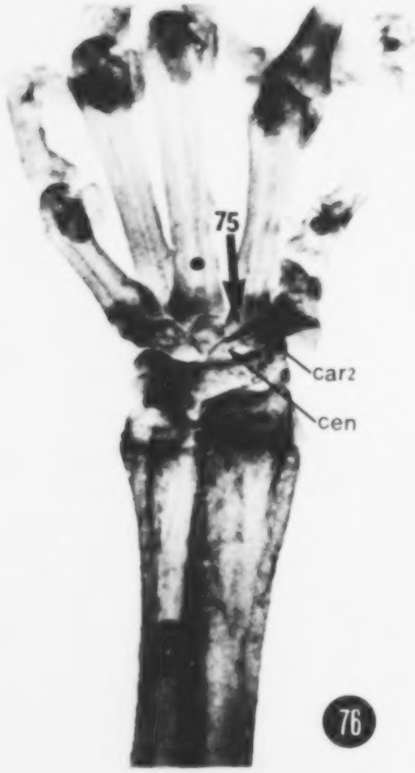

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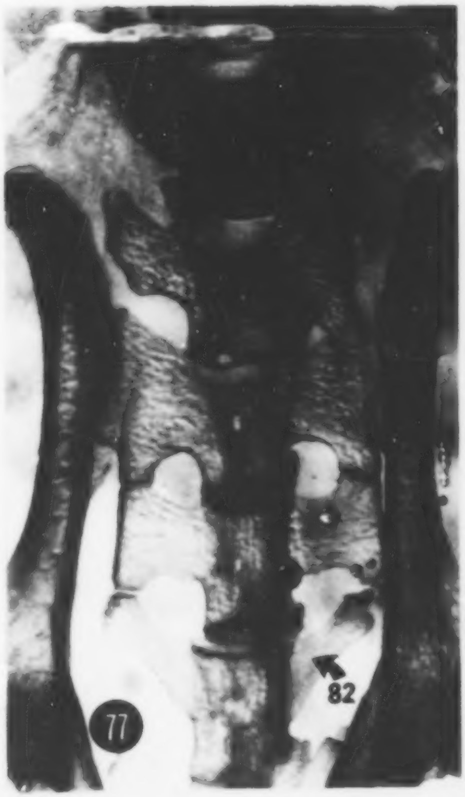

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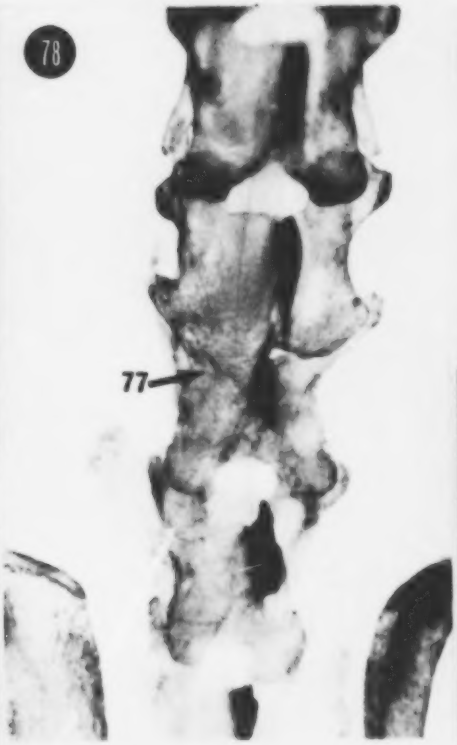

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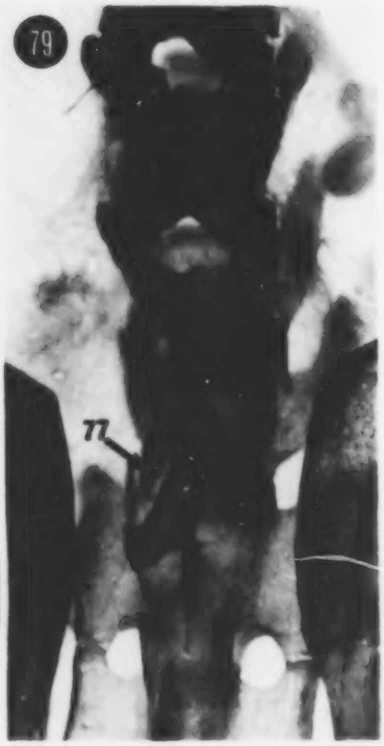

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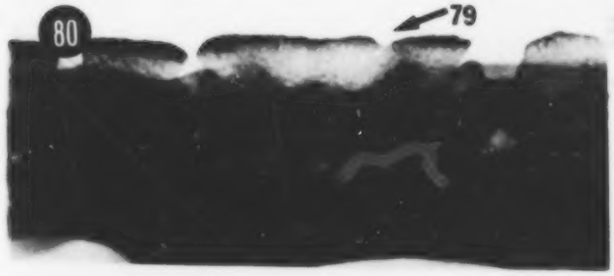
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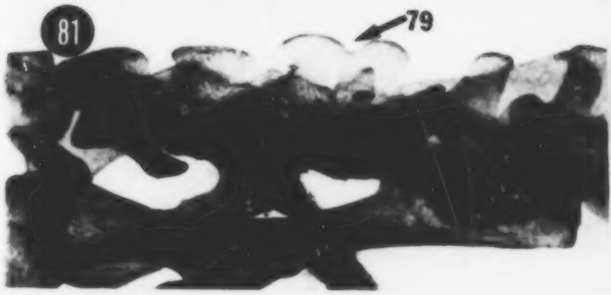
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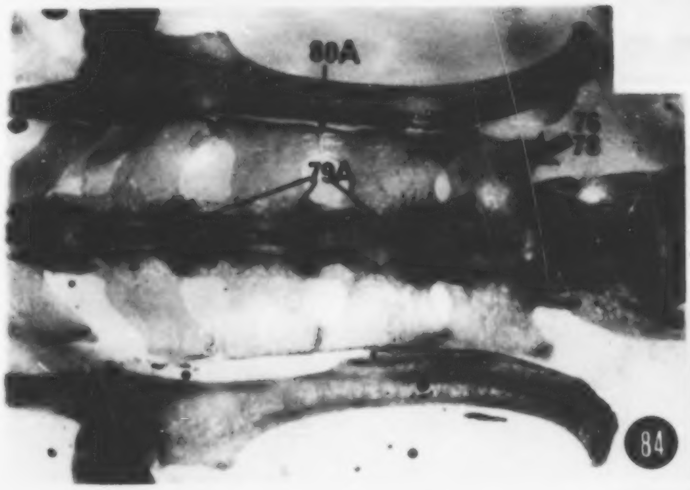
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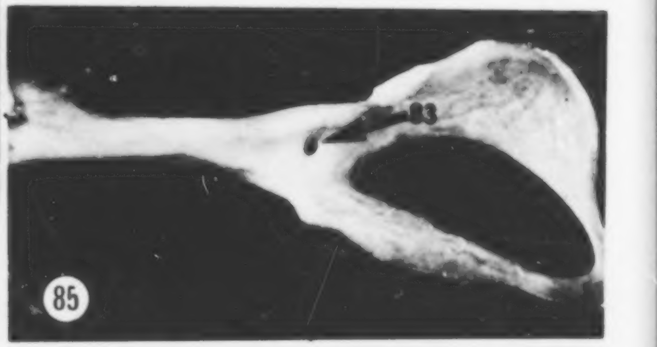
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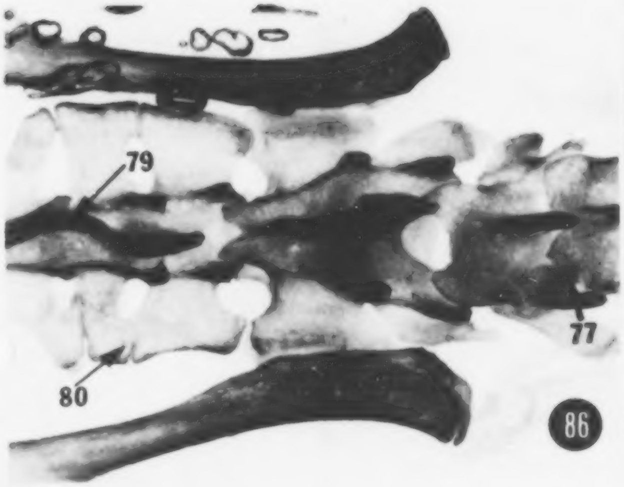
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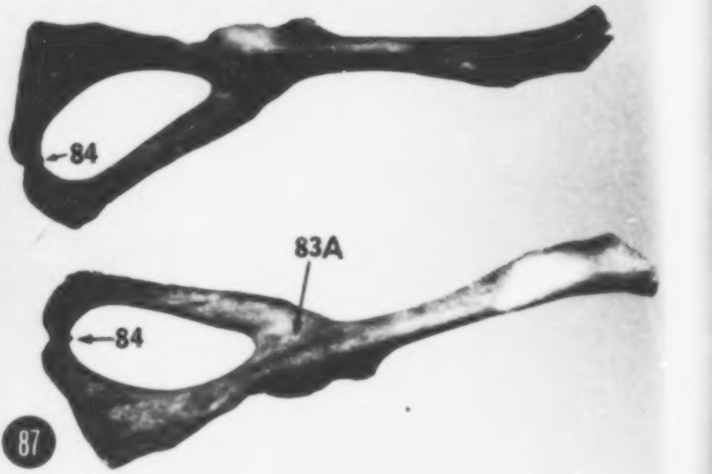
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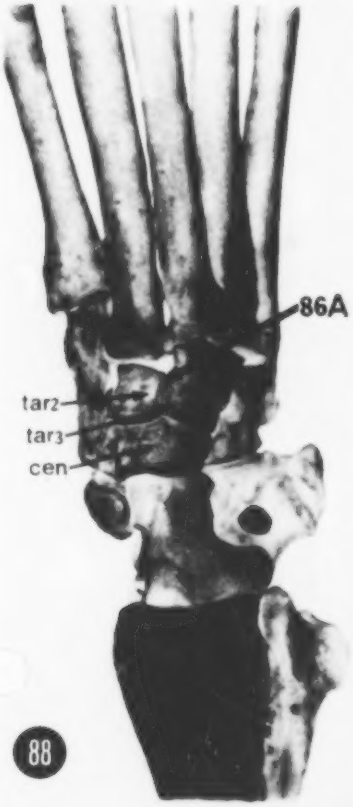
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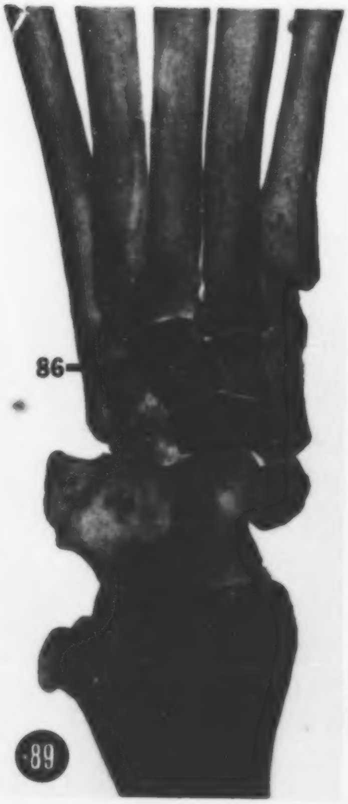

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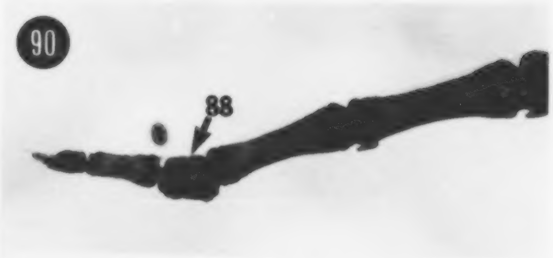

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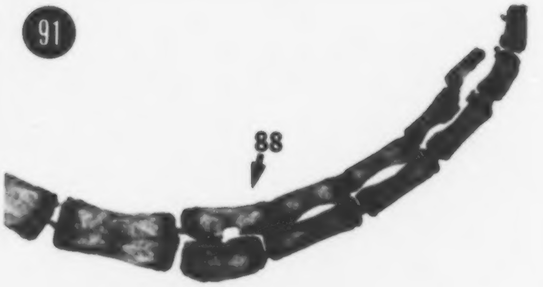

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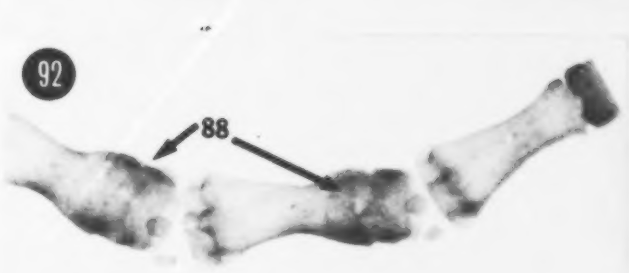

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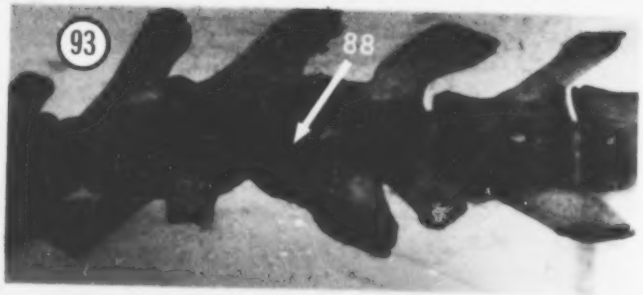

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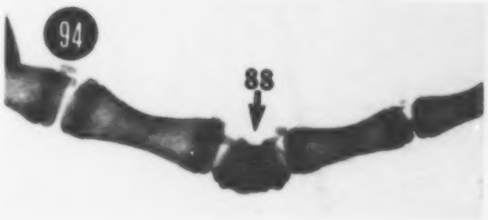

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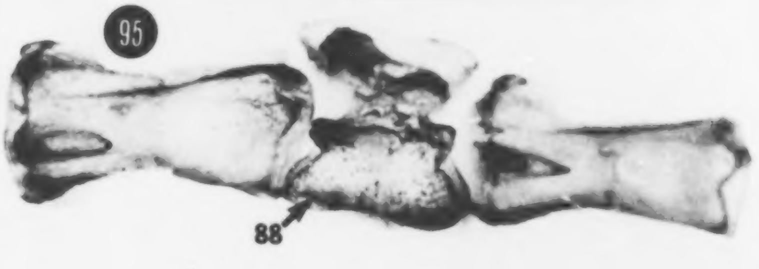

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Figures 96-109. Histograms of Frequencies of Occurrence and Differences in Frequency from Untreated and Vehicle for a Number of Skeletal Variants which are Sensitive or Important Indicators of Prenatal Exposure to a Variety of Compounds.

Legend:

In the histograms showing differences, the bars above the line indicate cases where the experimental group shows a higher frequency than the Untreated (B) or Vehicle-Treated (C) group. Those bars below the line indicate a reduced frequency of occurrence in the experimental group. The groups are as follows.

TFLT (Trifluralin/2,4,5-T)
1. Corn Oil-Veh(B) or UNTD(C), 2. 245T-L, 3. 245T-H, 4. Trifluralin

CAPT (Captan/Thalidomide)
1. CMC-Vehicle(B) or UNTD(C), 2. Thal-L, 3. Thal-H, 4. Captan

MNBD (Maneb/Decamethrin)
1. Corn Oil Veh(B) or UNTD(C), 2. Decamethrin, 3. CMC-Vehicle(B) or UNTD (C), 4. Maneb

TRBL, TRBL2 (first and second Trypan Blue Series)
1. 0.9% NaCl Veh (B) or UNTD(C), 2. 0.37T, 3. .075T, 4. 0.15T, 5. 0.30T

ACZM, ACZM2 (first and second Acetazolamide series)
1. Day 8 Water Vehicle (B) or UNTD (C), 2. ACZM-L8, 3. ACZM-H8, 4. Days 9-11 Water Veh (B) or UNTD (C), 5. ACZM-L9-11, 6. ACZM-H9-11

BUDR, BUDR2 (first and second Bromodeoxyuridine series)
1. Day 7 Water Veh (B) or UNTD (C), 2. BUDR-L7, 3. BUDR-H7, 4. Day 8 Water Veh (B) or UNTD (C), 5. BUDR-L8, 6. BUDR-H8; in the BUDR2 only, 7. Day 9 Water Veh (B) or UNTD (C), 8. BUDR-L9, 9. BUDR-H9

CORT (Cortisone Acetate)
1. 0.85% NaCl Veh (B) or UNTD (C), 2. .15 CORT, 3. .3 CORT, 4. .6 CORT, 5. 1.2 CORT

DPH (Diphenylhydantoin)
1. 0.05% NaOH Veh (B) or UNTD (C), 2. 6.25 DPH, 3. 12.5 DPH, 4. 25 DPH, 5. 50 DPH

See Table 2 for description of the treatments.

Figure 96.

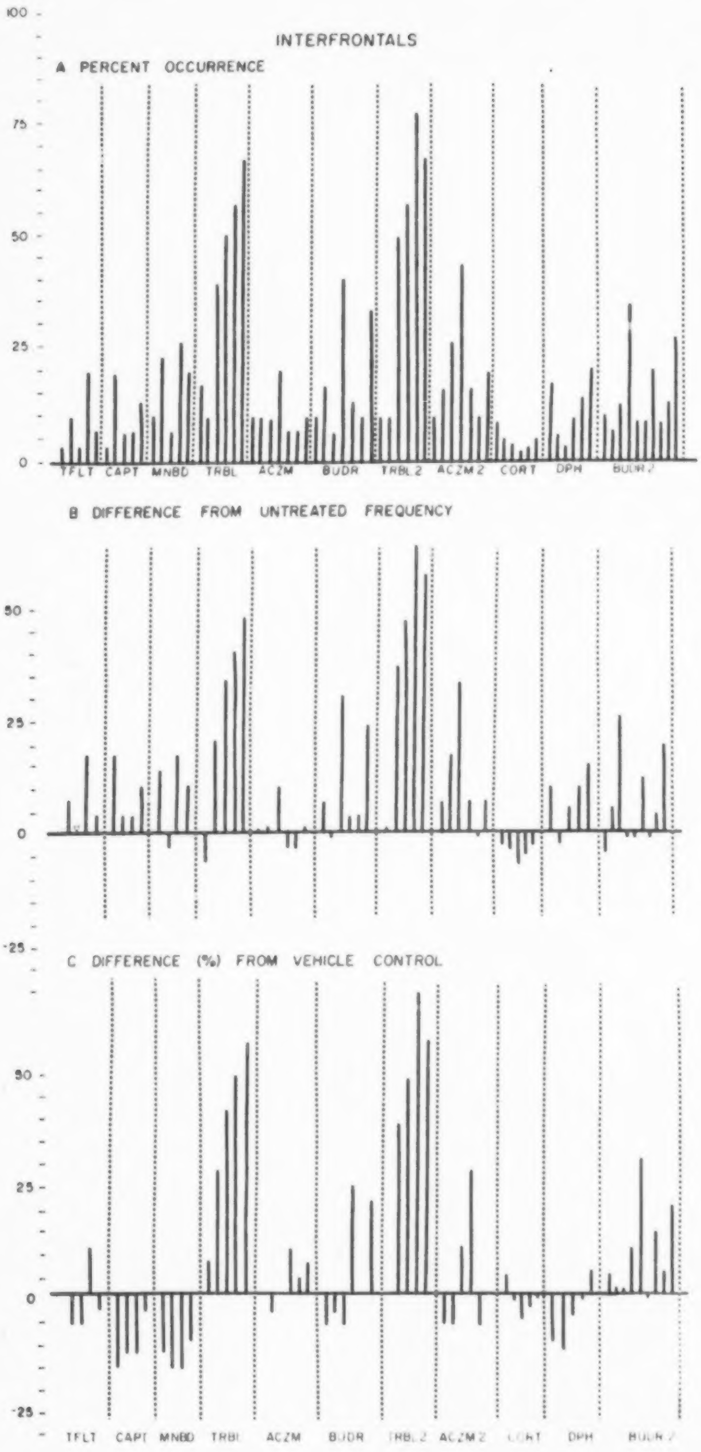
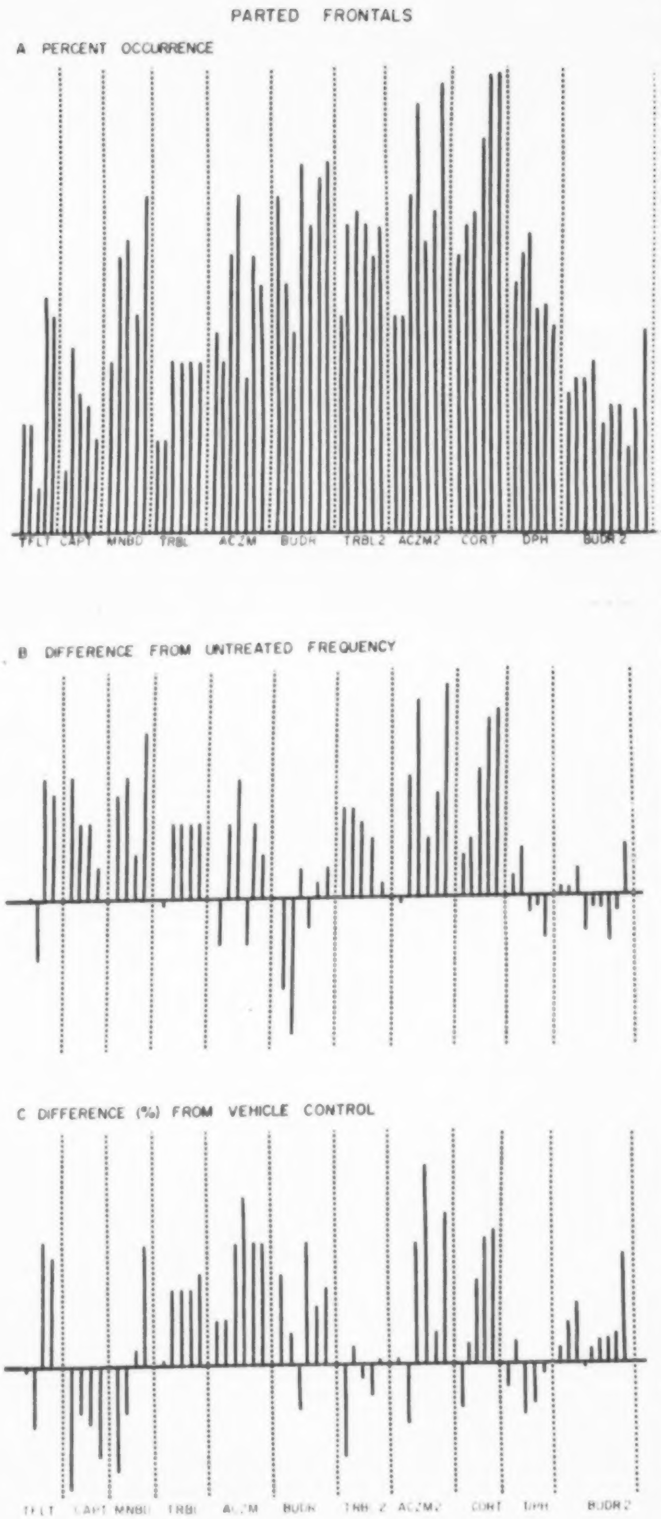


Figure 97.



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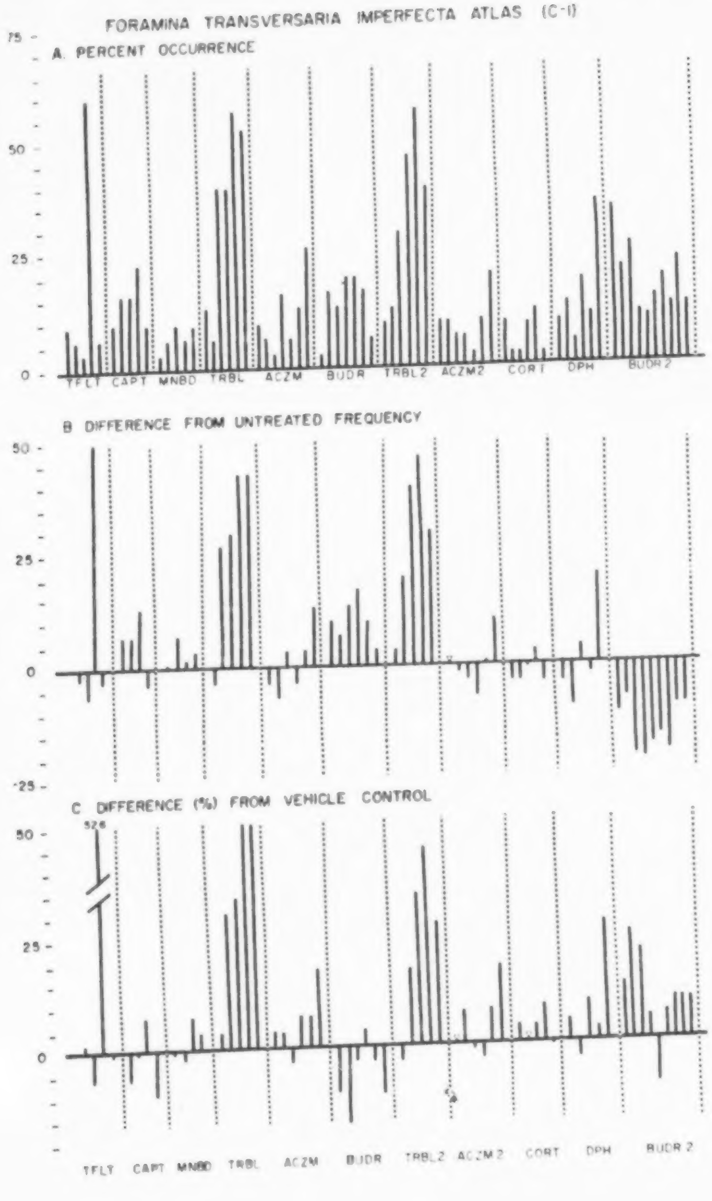

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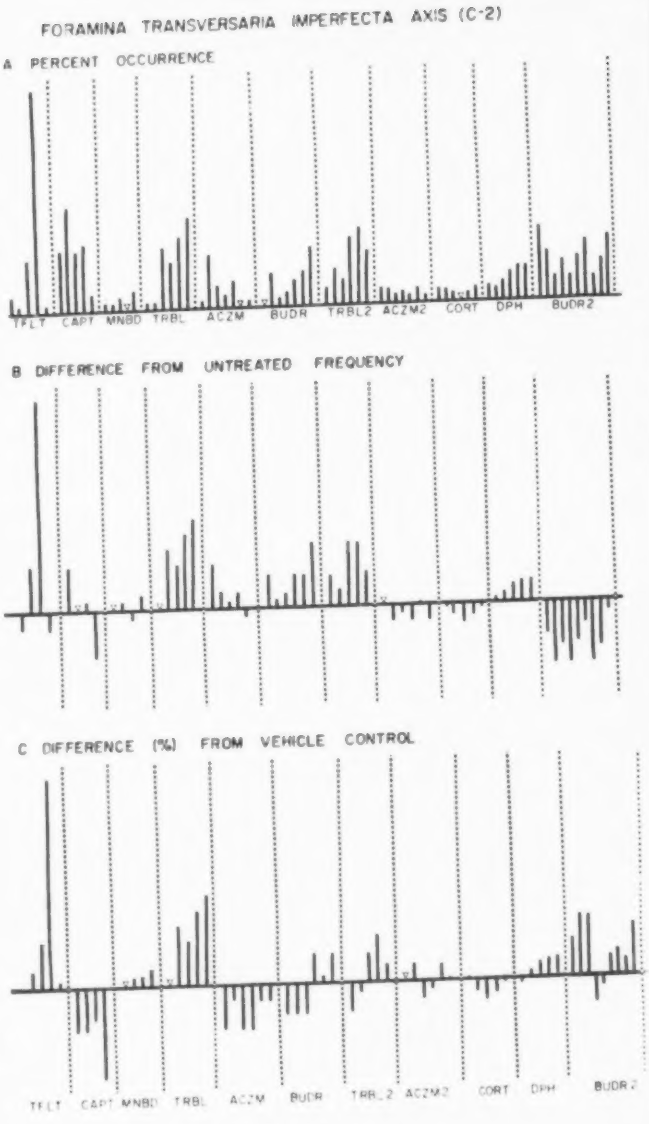

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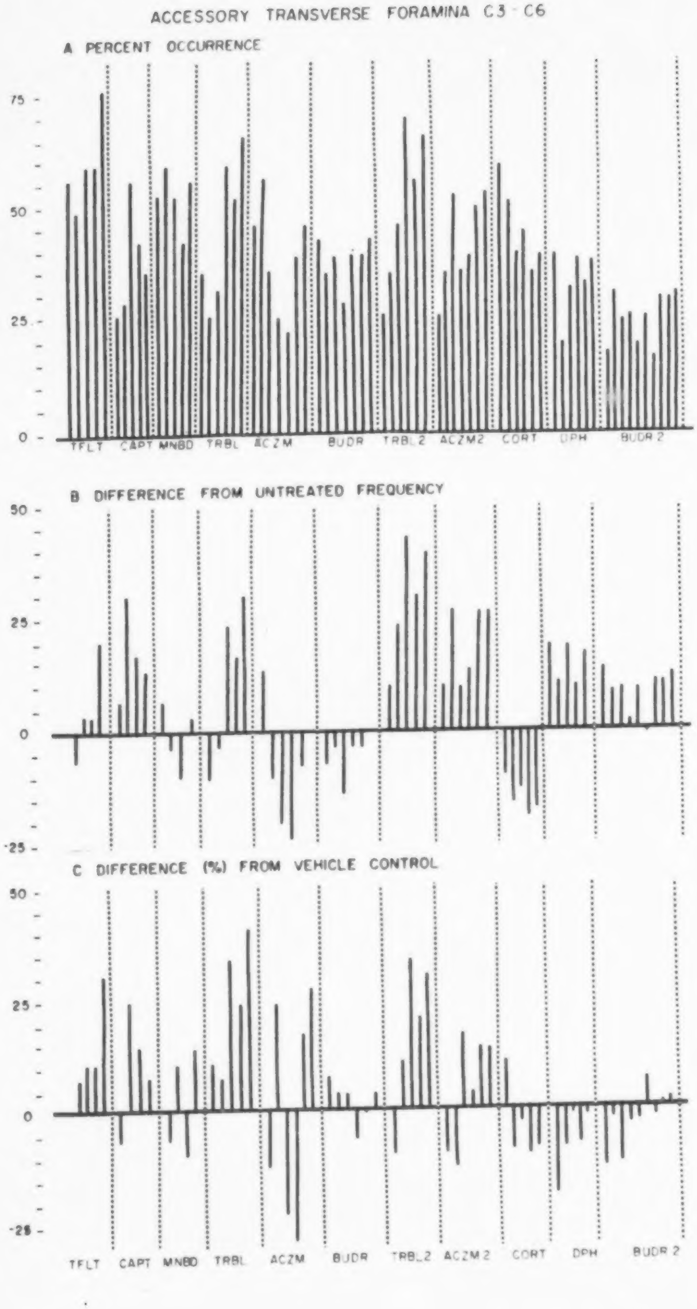

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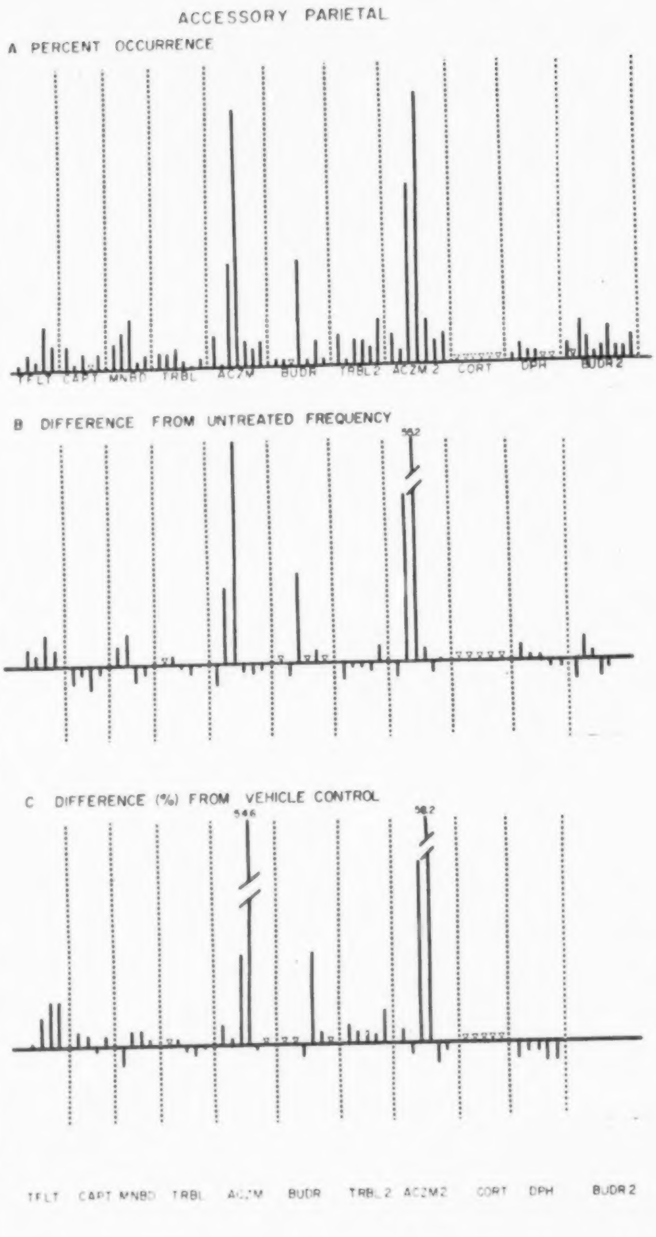

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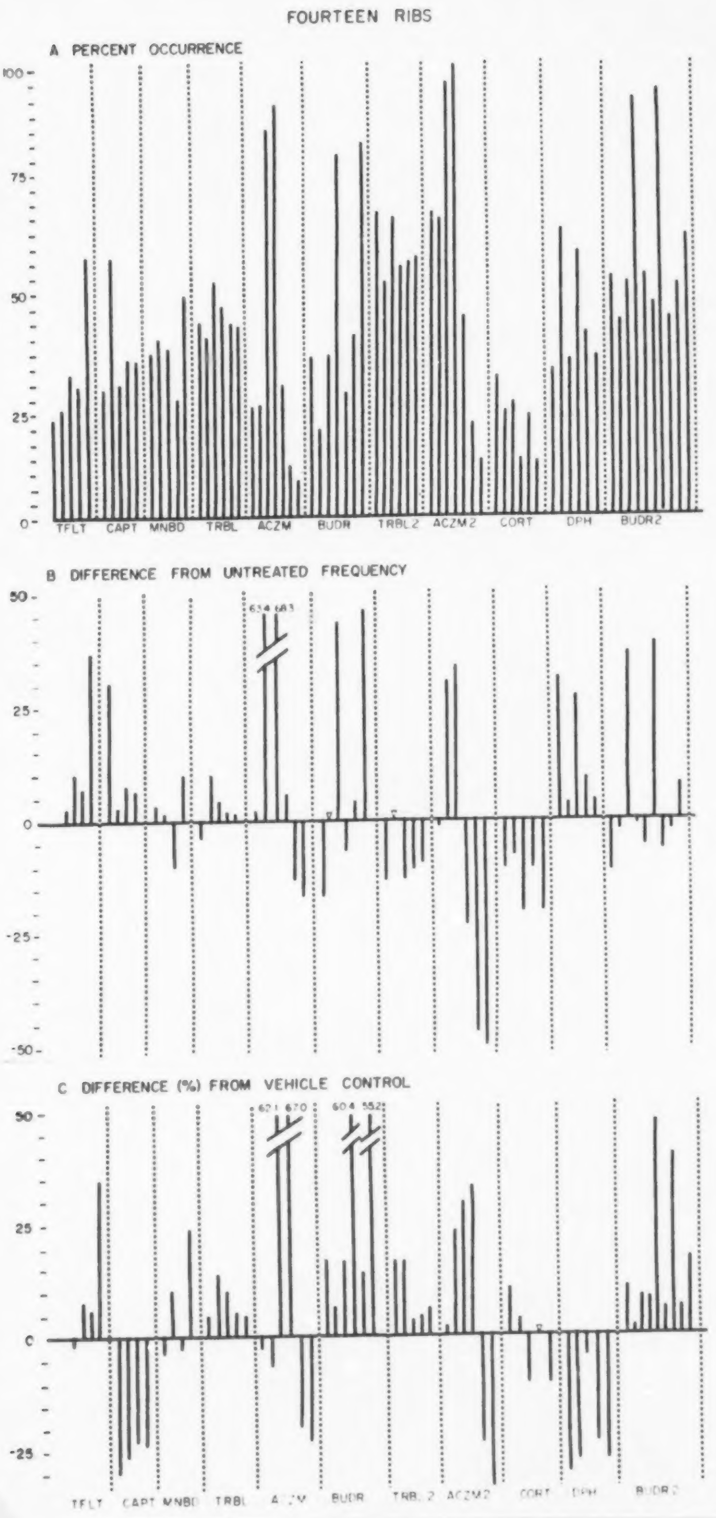

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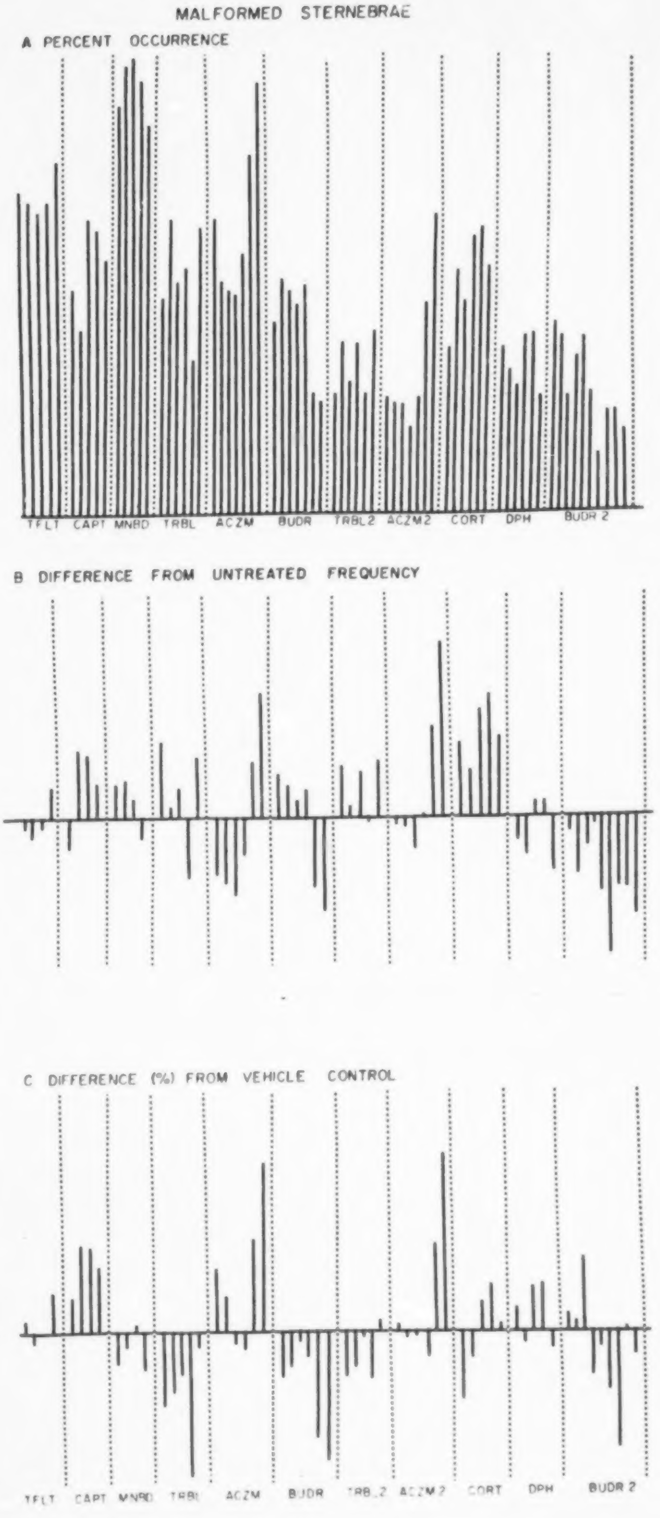

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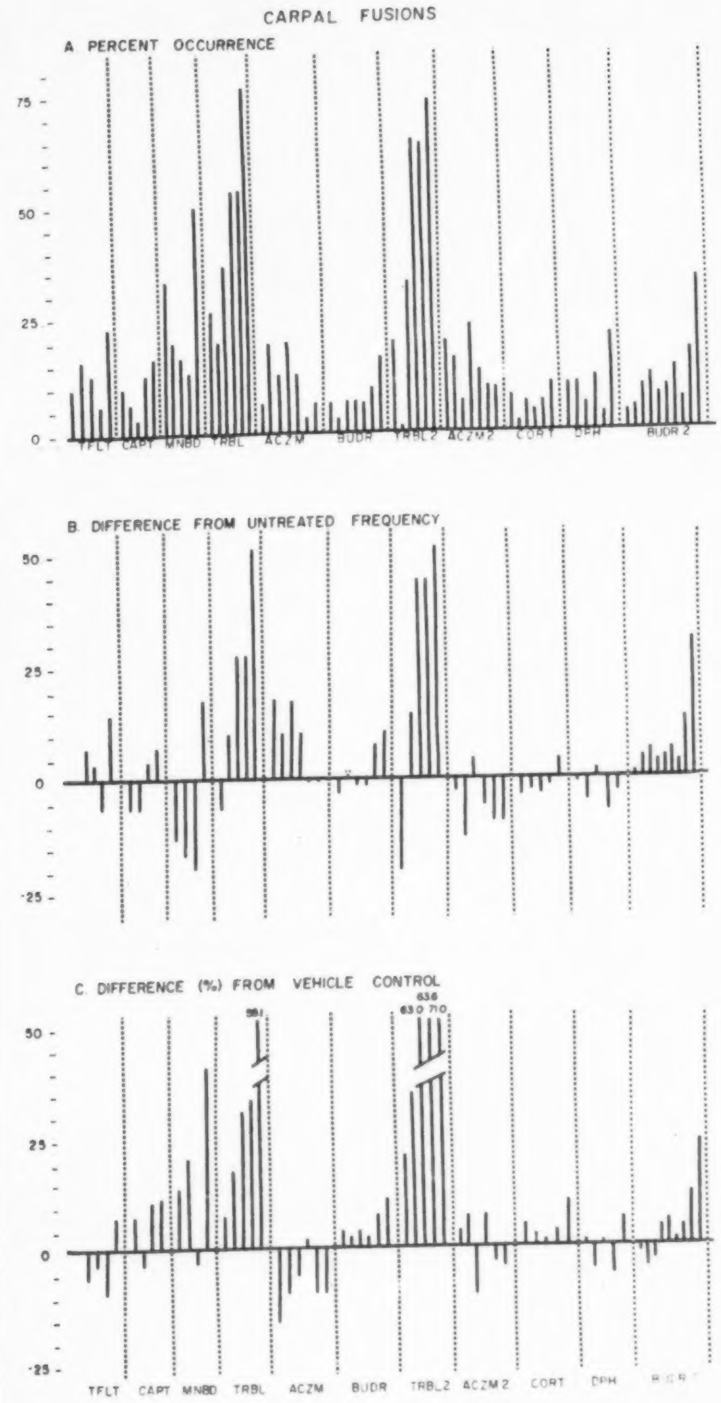

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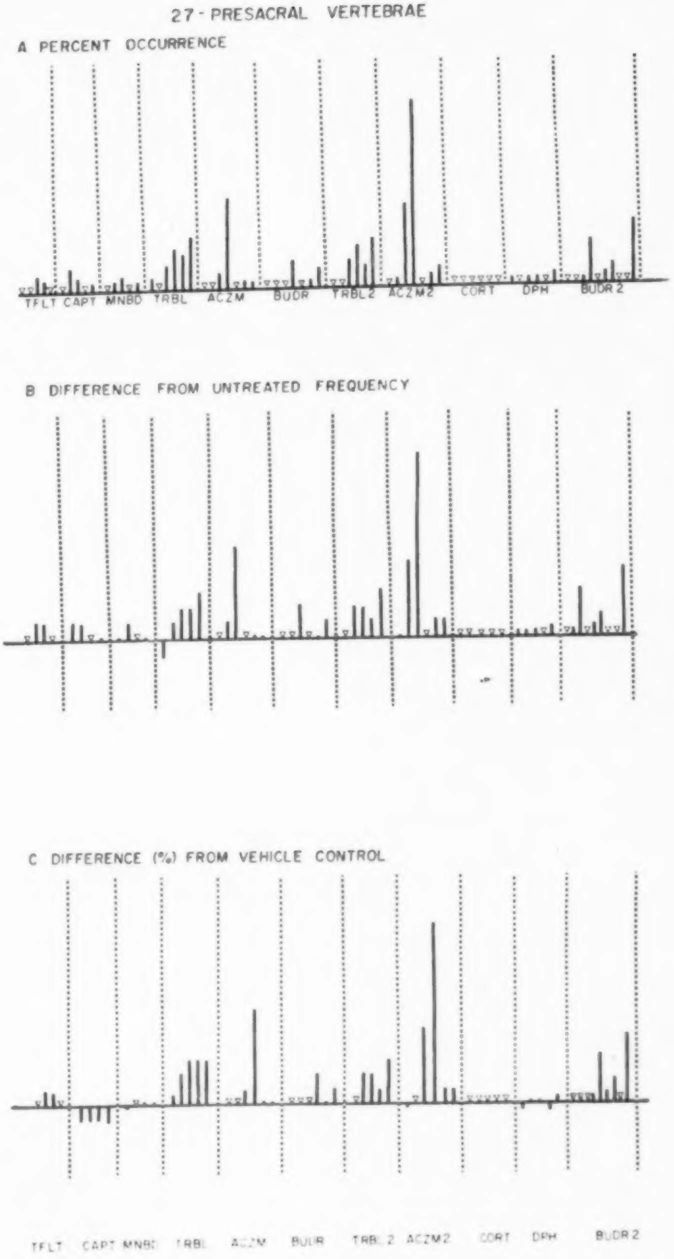

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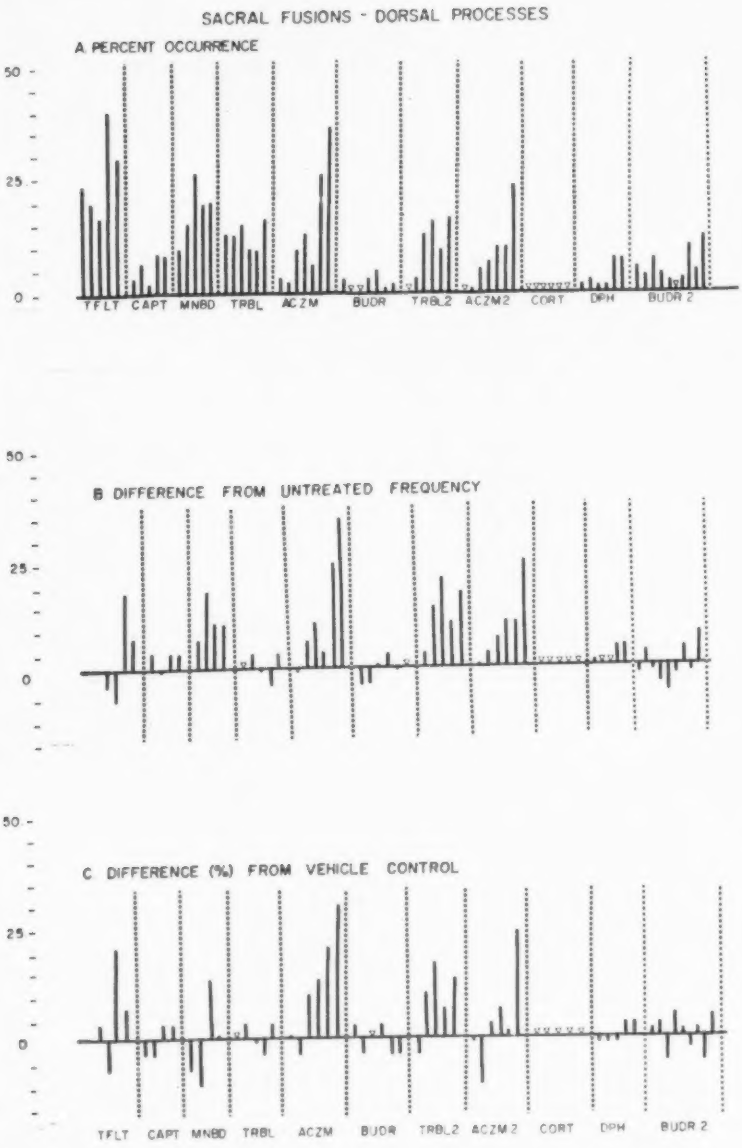

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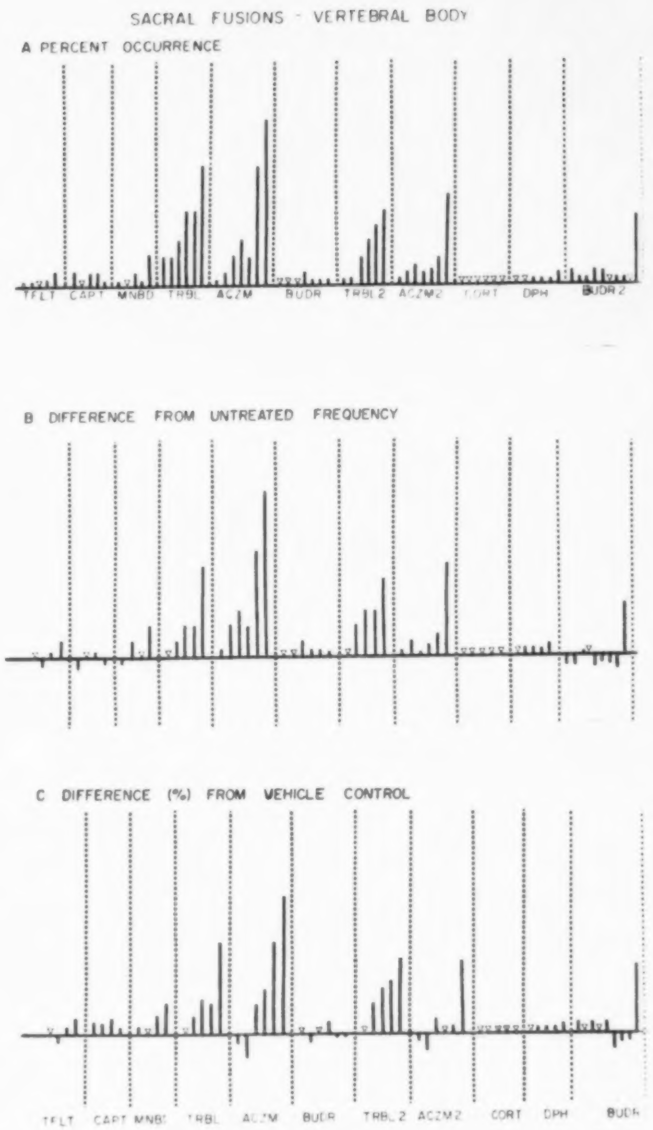

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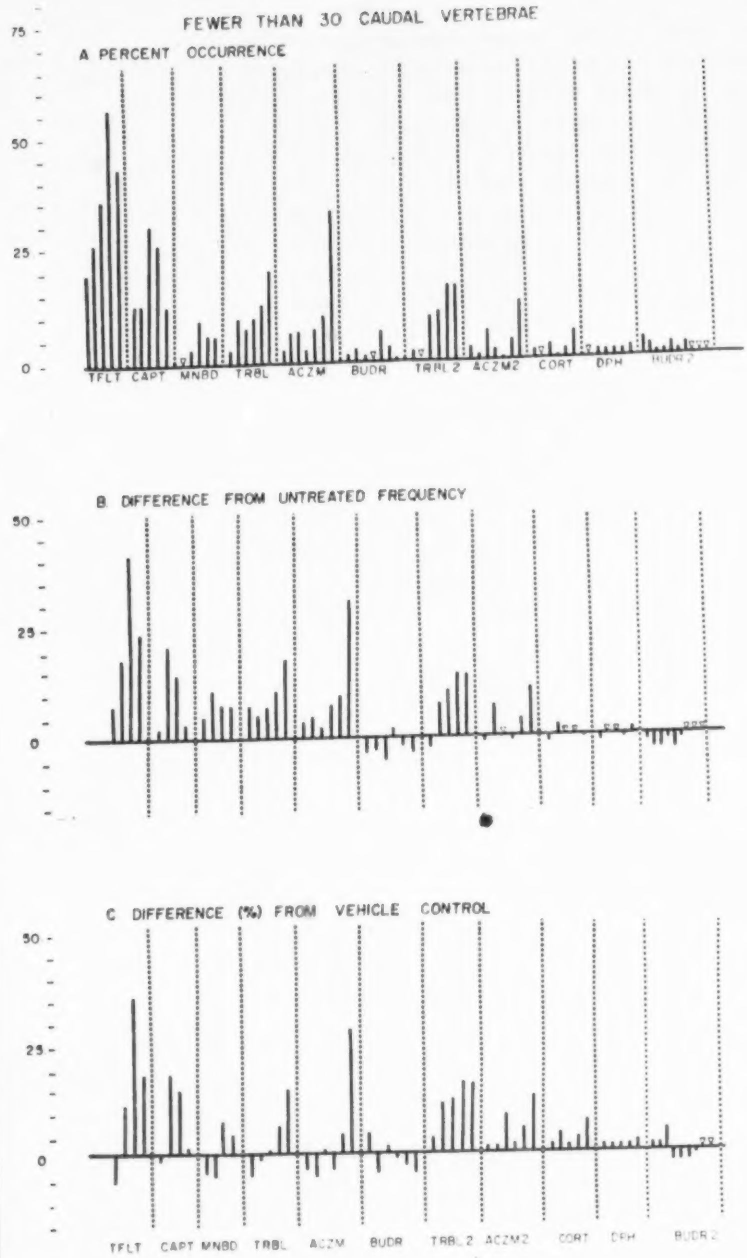


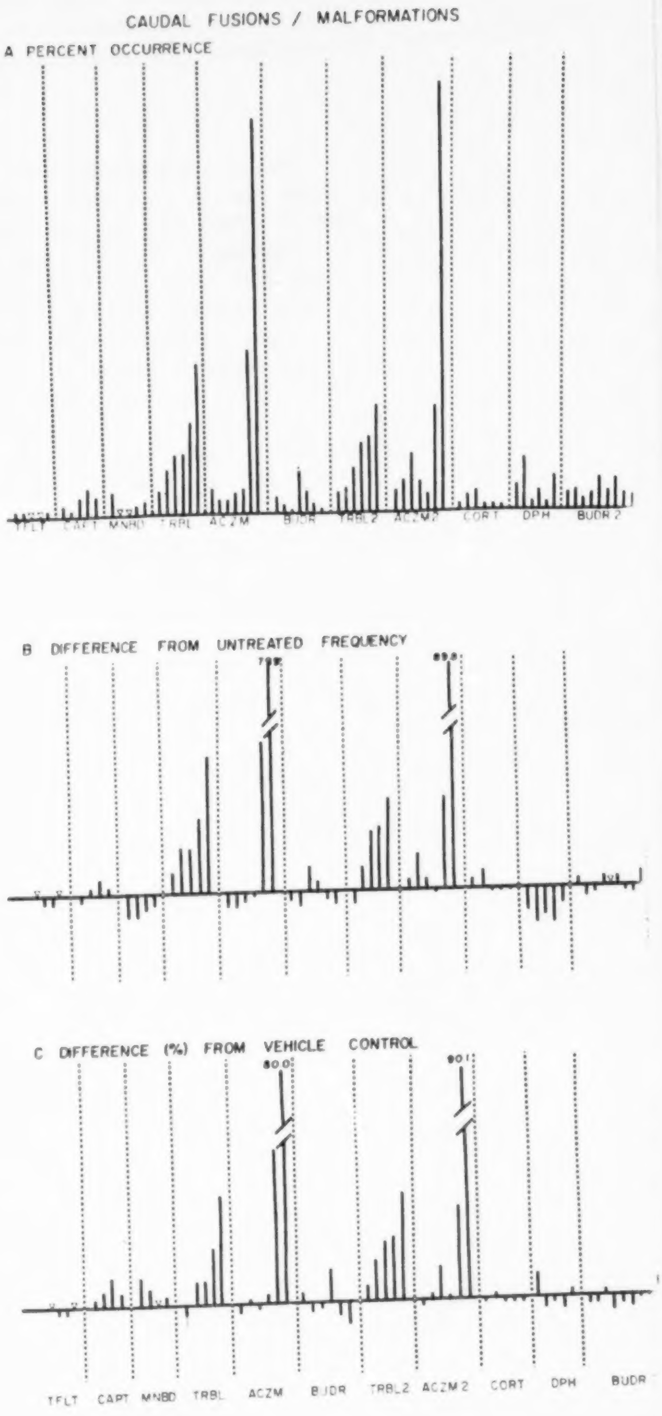
FEWER THAN 30 CAUDAL VERTEBRAE

A PERCENT OCCURRENCE

B. DIFFERENCE FROM UNTREATED FREQUENCY

C DIFFERENCE (%) FROM VEHICLE CONTROL

Figure 109.



CAUDAL FUSIONS / MALFORMATIONS

A PERCENT OCCURRENCE

B DIFFERENCE FROM UNTREATED FREQUENCY

C DIFFERENCE (%) FROM VEHICLE CONTROL

Figures 110-112. Differences in frequency of occurrence between experimental and untreated groups of CD-1 mice for all eighty-eight variants in three sets of replicated experiments.

Three compounds (trypan blue, acetazolamide, bromodeoxyuridine) were each tested twice. The histograms are graphic representations of replicability of the effects on the adult skeleton of prenatal exposure to the compound under investigation for all 88 variants. Variant numbers correspond with those in Table 2. All of the graphs are drawn to the same scale. As a point of reference, on Figure 110 variant #14-Interfrontal, in .037% Trypan Blue series 1 represents a difference from untreated of 20%. Significant differences ($P<.01$) from untreated for each variant, based on t-tests of arcsin-square root transformed frequencies are indicated by a star and the variant number at the top of the appropriate bar.

Figure 110. A comparison of two series of experiments with trypan blue. Differences between experimental and untreated in frequencies of occurrence of all eighty-eight variants.

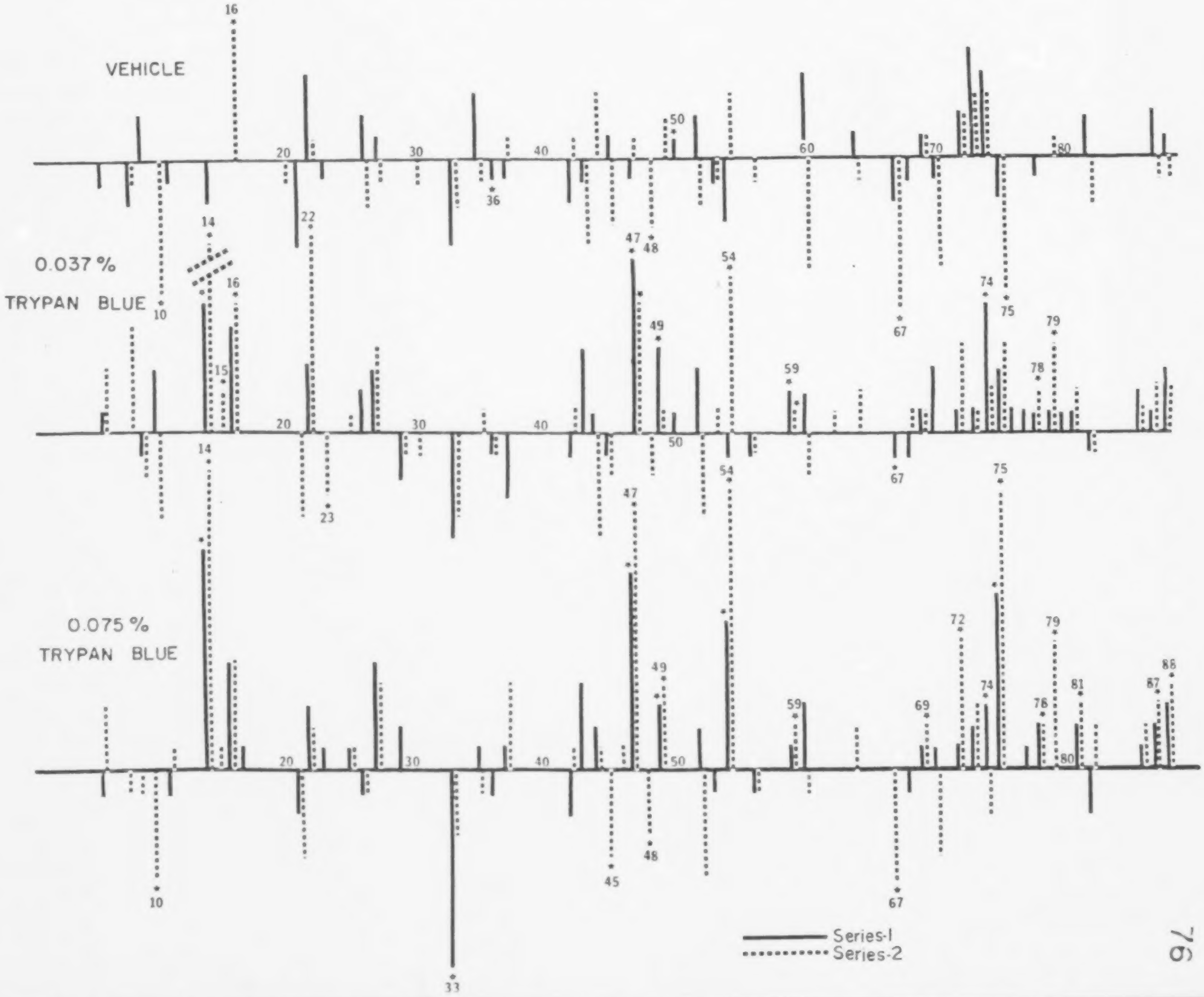


Table 110. Continued

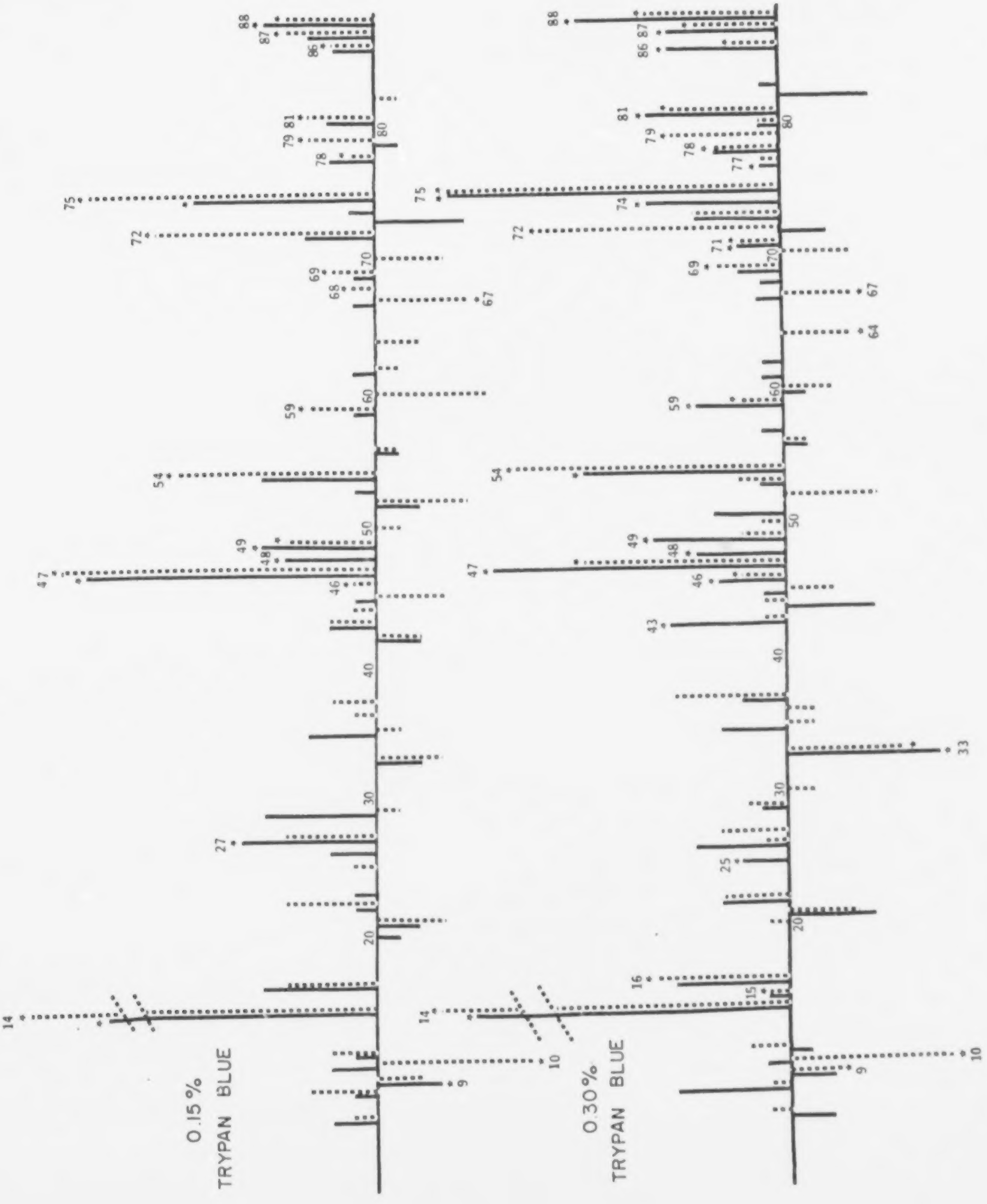


Figure 111. A comparison of two series of experiments with acetazolamide. Differences between experimental and untreated in frequencies of occurrence of all eighty eight variants.

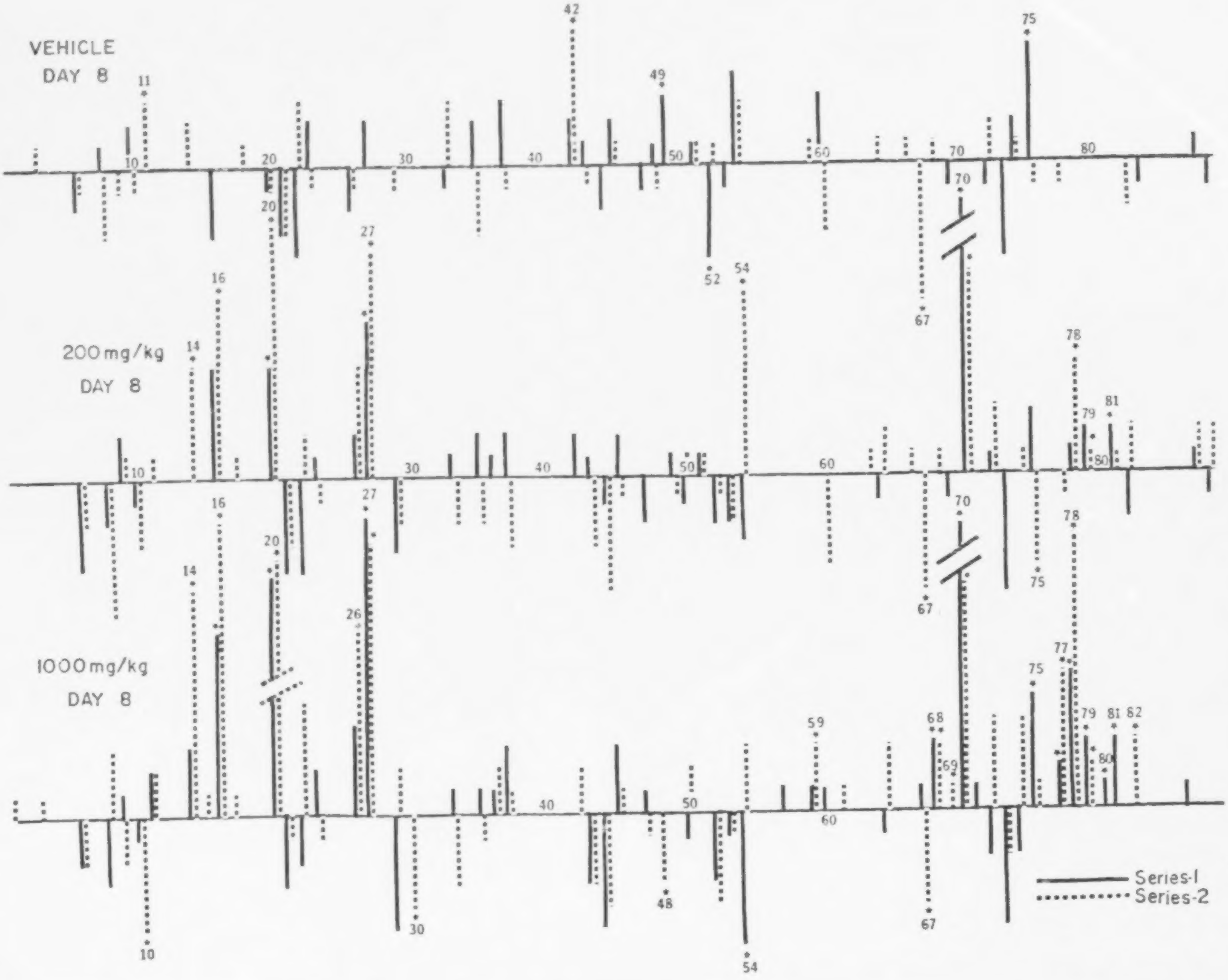


Figure III. Continued

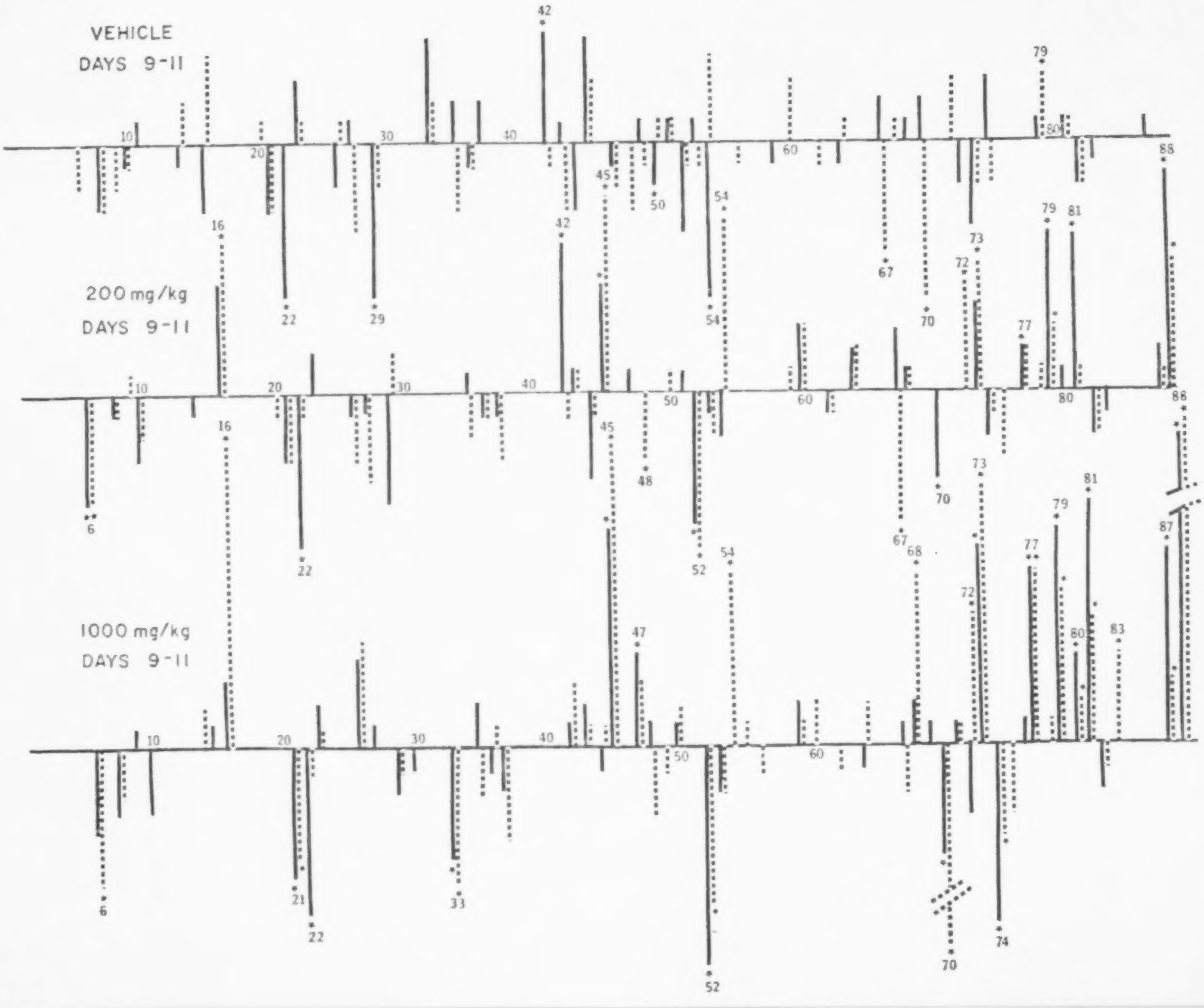


Figure 112. A comparison of two series of experiments with bromodeoxyuridine. Differences between experimental and untreated in frequency of occurrence of all eighty-eight variants.

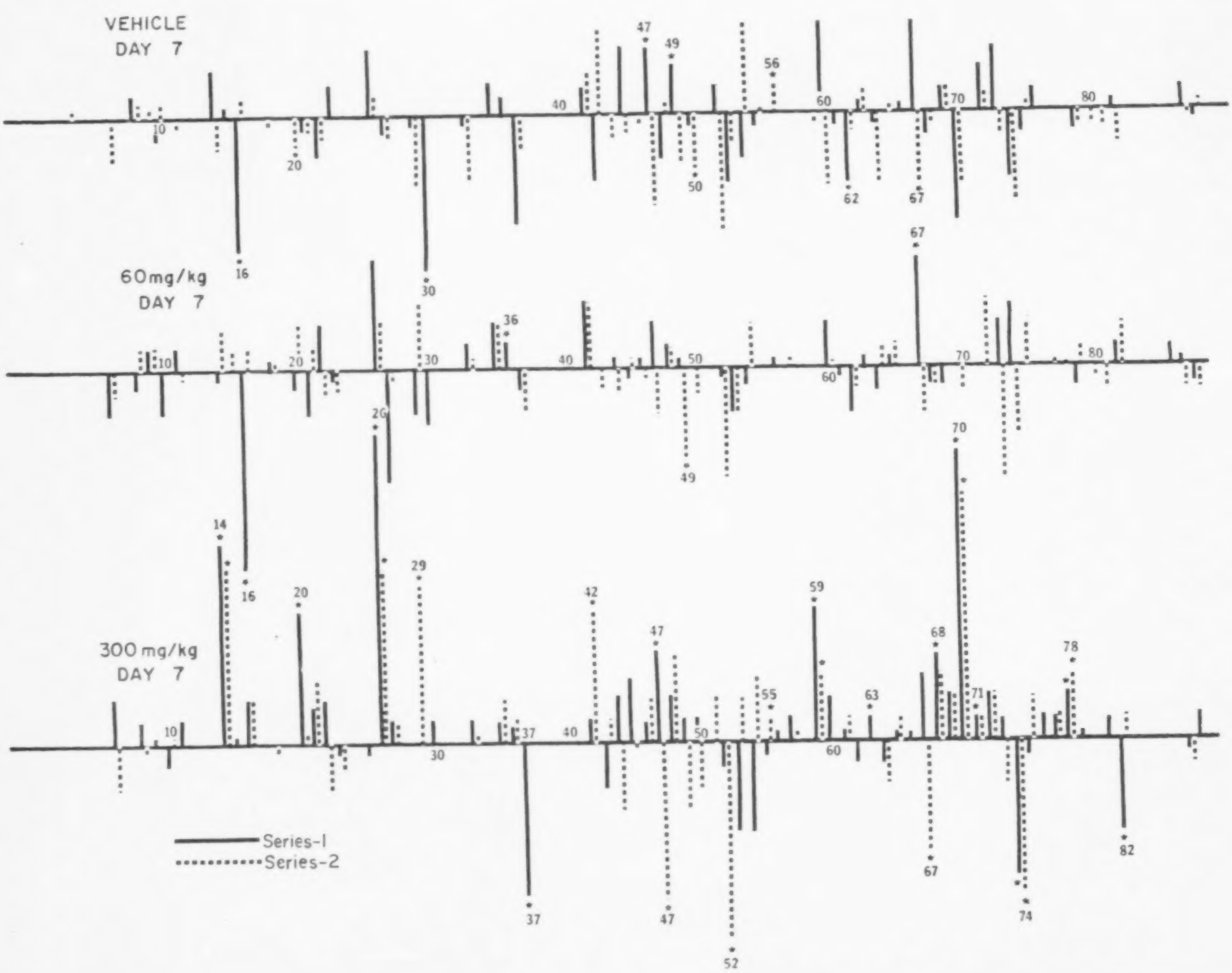
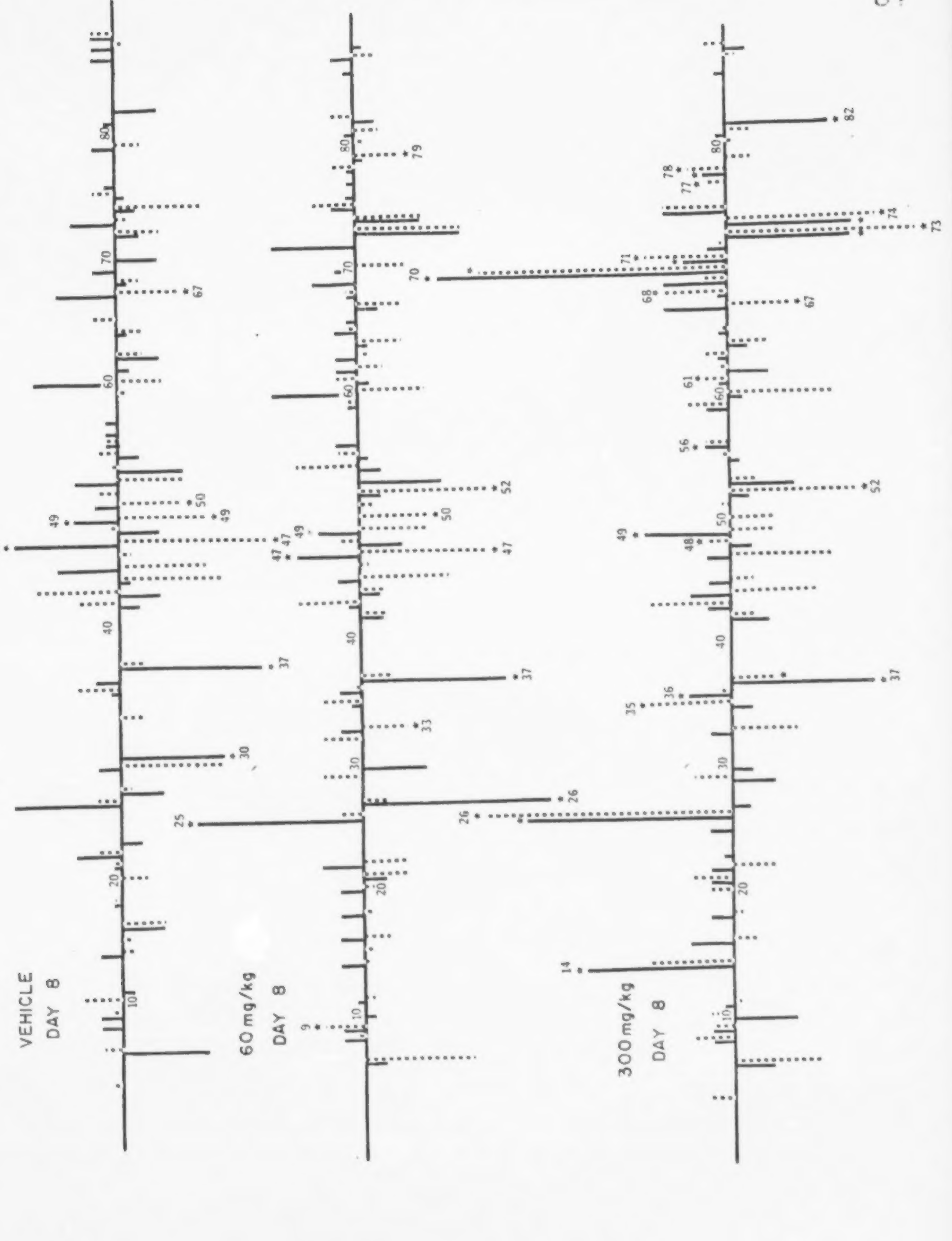


Figure 112. Continued



Appendix A. Vital Statistics for Each Experimental Series

The tables which follow describe the initial results of treatments and the perinatal consequences. In all experimental series non-productive females were autopsied to determine whether they had resorbed litters or were not pregnant. Stillbirth is defined as including pups which died within 24 hours of birth since litters were typically examined the morning after they were born. The number of dead pups is likely an underestimate since not all would be recovered. Upon examination, live pups were weighed together and an average weight calculated. The Mean Birth Weight in the tables is an average of the average for each litter and the number used for calculation of the Standard Error was the number of litters. For details of each treatment refer to Table 2.

Table A1. Vital Statistics in Maneb/Decamethrin Experiments

Parameter	UNTD	CMC	COC	Maneb-1500[a]	Maneb-1200	DMT
No. Females Mated	12	18	20	16	36	21
Maternal Mortality	0	0	1	6[b]	7[c]	0
Litters Born (Live)	10	11	13	2	13	15
Litters Stillborn	0	1	4	1	6[d]	1
Litters Resorbed	0	0	0	0	0	0
Females Not Pregnant	2	6	2	7	10	5
Mating Efficiency (Live Litters/No. Mated)	.83	.61	.65	.13	.36	.71
No. Pups Born	109	116	135	11	112	166
No. Pups Stillborn	0	10	26	24	51	5
Mean Litter Size ± S.E. at Birth (Live)	10.9±1.16	10.6±.82	10.4±.89	5.5±3.5	8.6±.77	11.1±.60
Mean Birth Weight ± S.E. Pup (Live)	1.77±.11	1.66±.03	1.66±.11	2.03±0	1.68±.06	1.68±.05

Legend:

a. The 1500 mg/kg/day Maneb treatment was discarded as being too toxic, and the data are provided here for completeness.
b. All were autopsied, five were pregnant, but fetuses were severely retarded developmentally.
c. Four were autopsied, all four were pregnant, some litters were retarded.
d. Several of the stillborn litters seemed to consist of premature pups.

Table A2. Vital Statistics in the First Series of Trypan Blue Experiments With CD-1 Mice.

Parameter	UNTD	VEH	0.037TB	0.075TB	0.15TB	0.3TB
No. Females Treated	16	16	19	18	24	28[a]
Maternal Mortality	0	0	0	0	0	1
Litters Born (Live)	15	14	13	14	15	19
Litters Stillborn	0	1	3[b]	2[b]	5[c]	3
Litters Resorbed	0	1	1	0	1	3
Females Not Pregnant	1	0	2	2	3	2
Mating Efficiency (Live Litters/ No. Mated)	94%	88%	68%	78%	63%	68%[a]
No. Pups Born	147	129	107	110	99	95
No. Pups Stillborn						
in Live Litters	2	7	3	2	2	19
in Dead Litters	0	7	20	8	28	9
Mean Litter Size ±S.E. (Live)	9.8±1.15	9.2±.89	8.2±1.04	7.9±.50	6. ±1.06	5.0±.53
Mean Birth Weight ±S.E./Pup (Live)	1.63±0.1	1.75±0.1	1.73±0.1	1.71±0.1	1.79±0.	1.81±0.1
Litters Weighed	(9)	(13)	(11)	(10)	(12)	(15)
Malformations	d	e	d	d	e,f	e,g

Legend:

a. One female died in the isolation cage on day 17 post coitus. She was not pregnant.
b. Saw liveborn pups in one litter. Dam was continually running around the cage, possibly suffering a middle ear disturbance. Litter died within 24 hours.
c. Saw liveborn pups in two litters; entire litter dead within 24 hours.
d. No malformations observed.
e. One or two runts which did not survive to sacrifice date.
f. One dead pup in a live litter was exencephalic.
g. Seven pups with tail kinks or curled tails, distributed over 5 litters.

Both trypan blue series have recently been reevaluated as the data are being worked up for publication.

Table A3. Vital Statistics in the Second Series of Trypan Blue Experiments with CD-1 Mice.

	UNTD[a]	VEH	.037T	.075T	.15T	.3T
No. Females Treated	27	14	15	20	21	24
Maternal Mortality	0	0	0	0	0	0
Litters Born (Live)	22	13	12	12	19	20
Stillborn Litters		1[b]	1	2	2	0
Resorbed Litters	0	0	0	2	1	1
Females Not Pregnant	4	0	2	4	1	3
Mating Efficiency (Live Litters/ No. Mated)	81%	93%	80%	60%	90%	83%
No. Pups Born	199	138	77	83	91	130
No. Pups Stillborn						
in Live Litters	1	0	2	5	5	4
in Dead Litters	5	7	1	4	1	0
MLS±S.E. (Live)	9.0 ±.51	10.6±.49	6.4±.72	6.9±.76	5.2±.63	6.5±.51
Mean Birth Weight per Live Pup (grams 1±S.E.)	1.78±.04	1.62±.03	1.75±.05	1.80±.05	1.82±.04	1.72±.04
No. Litters Weighed	(17)	(13)	(12)	(12)	(17)	(20)
Malformations	c	d	d,e	c	f	g

Legend: Both Trypan Blue series have recently been reevaluated as the data are being worked up for publication.

a. The UNTD are the same group as in Table 6, with some additions.
b. Saw liveborn pups in one litter. Dam was continually running around the cage, possibly suffering a middle ear disturbance. Litter died within 24 hours
c. No malformations observed.
d. One or two runts which did not survive to sacrifice date.
e. Two pups with curled tails, one in each of two litters.
f. Absence of tail on one dead pup.
g. One pup with curled tail.

Table A4. Vital Statistics in the First Series of Acetazolamide Experiments

Parameter	UNTD	VEH-8	ACZM-L8	ACZM-H8	VEH-9X3	ACZM-L9X3	ACZM-H9X3
No. Females Treated	12	15	17	19	15	16	14
Maternal Mortality	0	0	0	0	0	0	0
Litters Born (Live)	12	11	13	17	12	14	13
Litters Stillborn	1	1	1	2	1	1	0
Litters Resorbed	0	0	0	0	0	0	0
Females Not Pregnant	0	3	3	0	2	1	1
Mating Efficiency (Live Litters/No. Mated)	100%	73.3%	76.5%	89.5%	80.0%	87.5%	92.2%
No. Pups Born	116	102	128	149	126	134	137
No. Pups Stillborn	9	10	17	26	6	15	1
Mean Litter Size ± S.E. at Birth (Live Pups)	9.67±.94	9.27±.86	9.85±.90	8.76±.70	10.5±.91	9.6±.72	10.5±.72
Mean Birth Weight/ Pup (Live)	1.66±.05	1.69±.03	1.65±.04	1.69±.06	1.69±.06	1.65±.04	1.54±.04
No. Litters Weighed	(11)	(11)	(13)	(17)	(8)	(14)	(13)

Table A5. Vital Statistics in the Second Series of Acetazolamide Experiments.

Parameter	UNTD[a]	VEH-8	ACZM L-8	ACZM H-8	VEH-9X3	ACZM-L9X3	ACZM-H9X3
No. Females Treated	21	20	15	25	14	21	17
Maternal Mortality	0	1	0	0	1	0	4
Litters Born (Live)	17	13	11	17	11	14	10
Litters Stillborn	2	5	2	4	1	1	1
Litters Resorbed	0	0	1	3	0	2	1
Females Not Pregnant	2	1	1	1	1	4	1
Mating Efficiency (Live Litters/ No. Mated)	81%	80%	73%	68%	79%	67%	59%
No. Pups Born	143	104	119	116	87	129	87
No. Pups Stillborn	17	42	11	33	7	1	8
Mean Litter Size at Birth ± S.E. (Live Pups)	8.4±.51	8.0±.88	10.8±.58	6.8±.76	7.9±.78	9.2±.60	8.7±.99
Mean Birth Weight Per Live Pups (gm±S.E.)	1.78±.04	1.70±.06	1.65±.06	1.60±.04	1.72±.08	1.62±.04	1.52±.04
No Litters Weighed	(14)	(9)	(8)	(10)	(10)	(14)	(10)

Legend:

a = The UNTD are the same group as in Table A3.

Table A6. Vital Statistics in the First Series of Bromodeoxy-uridine Experiments

	UNTD	VEH-7	BUDR-L7	BUDR-H7	VEH-8	BUDR-L8	BUDR-H8
Females Treated	18	15	15	16	14	14	14
Maternal Mortality	0	0	0	0	0	0	0
Litters Born (Live)	17	13	14	14	14	13	13
Litters Stillborn and Resorbed (R)	1	1	1(R)	0	0	0	1
Females Not Pregnant	0	1	0	2	0	1	0
Mating Efficiency (Live Litters/ No. Mated)	94%	87%	93%	88%	100%	93%	93%
No. Pups Born	209	140	158	144	177	123	114
No. Pups Stillborn	3	2	1	7	2	1	24
Mean Litter Size at Birth ± S.E.	12.3±0.6	10.8±0.7	11.3±0.6	10.3±0.4	12.6±0.4	9.5±0.9	8.8±1.0
Mean Birth Weight (gm±S.E.)/ pup	1.62±.04	1.69±.04	1.59±.03	1.54±.04	1.59±.03	1.63±.04	1.66±.05

Table A7. Vital Statistics in the Second Series of Bromodeoxyuridine Experiments

Parameter	UNTD	Veh-7	L-7	H-7	Veh-8	L-8	H-8	Veh-9	L-9	H-9
No. Females Treated	22	17	18	21	19	18	29	20	19	20
Maternal Mortality	0	0	0	0	0	0	0	0	0	0
Litters Born Live	15	12	16	16	14	14	21	18	15	16
Litters Stillborn	3	1	1	1	3	1	3	1	0	0
Litters Resorbed	0	0	1	0	0	1	0	0	0	0
Females Not Pregnant	4	4	0	4	2	2	5	1	4	4
Mating Efficiency (Live Litters/ No. Mated)	68.2%	70.6%	88.9%	76.2%	73.7%	77.8%	72.4%	90.0%	79%	80%
No. Pups Born[a]	151	122	169	135	130	156	103	172	149	137
No. Stillborn[b]	23	8	12	15	29	5	74	2	1	1
Mean Litter Size at Birth ±S.E.	10.1±.75	10.1±.60	10.6±.48	8.4±.87	9.3±.78	11.1±.51	4.9±.65	9.6±.60	9.9±.72	8.6±.58
Mean Birthweight Pup ±S.E.[c]	1.66±.04	1.63±.04	1.60±.02	1.52±.04	1.66±.03	1.59±.03	1.66±.03	1.66±.03	1.70±.04	1.62±.04
No. Perinatal Dead With Exencephaly/ No. Examined	0/1	0/0	0/0	3/9	1/8	0/2	56/58	0/1	0/0	0/1

Legend: The large number of dead pups is a reflection of the high frequency of Exencephaly produced by these treatments.

a. This number represents the number alive at 1 day post natal, when the litters were examined.
b. This includes those pups which died in the birth process due to exencephaly and related disorders.

Table A8. Vital Statistics in the Cortisone Acetate Experiments

Parameter	UNTD	Vehicle	.15mg/.1cc	.3mg/.1cc	.62/.1cc	1.25mg/.1cc
No. Females Treated	15	16	19	20	19	26
Maternal Mortality	0	0	0	0	0	0
Litters Born Live	15	16	18	16	18	17
Litters Stillborn	0	0	0	1	0	7
Litters Resorbed	0	0	0	2	0	0
Females Not Pregnant	0	0	1	1	1	2
Mating Efficiency (Live Litters/ No. Mated)	100%	100%	95%	80%	95%	65%
No. Pups Born	163	160	183	186	157	104
No. Stillbirths	0	2	2	17	32	152
Mean Birth Weight/Pup	1.62±.77	1.62±.03	1.60±.02	1.49±.03	1.40±.03	1.33±.04
Mean Litter Size at Birth±S.E. (Live Pups)	10.9±.77	10.1±.37	10.2±.54	11.6±.53	8.7±.83	6.1±.94
No. Stillbirths With Cleft Palate/ No. Examined	0/0	0/0	2/2	2/8	24/32	92/116

Legend:

The large number of perinatal deaths (stillbirths) is a reflection of the high frequency of cleft palate in these pups. Among those pups alive at 1 day postnatal most survived to weaning. The preweaning loss in the various groups was 1,1,0,2,5, and 1, respectively.

Table A9. Vital Statistics in Diphenylhydantoin Experiments

Parameter	UNTD	Vehicle	6.25mg/kg	12.5mg/kg	25mg/kg	50mg/kg
No. Females Treated	19	22	15	17	19	19
Maternal Mortality	0	0	1	1	0	0
Litters Born Live	19	20	13	14	14	17
Litters Stillborn	0	0	1	2	1	1
Litters Resorbed	0	1	0	0	1	1
Females Not Pregnant	0	1	0	0	3	1
Mating Efficiency (Live Litters/ No. Mated)	100%	91%	87%	82%	74%	89%
No. Pups Born	197	187	116	126	144	164
No. Stillborn	1	4	9	15	7	7
Mean Litter Size at Birth ± S.E. (Live Pups)	10.4±.53	9.5±.51	8.9±.80	9.0±.96	10.3±.51	9.7±4.7
Mean Birthweight/ Pup (Live)	1.6±.03	1.6±.03	1.7±.05	1.7±.05	1.6±.03	1.6±.03

Table A10. Vital Statistics in 2,4,5-T/Trifluralin Experiments

	UNTD	Corn Oil	245T-L	245T-H	TFL
No. Females Treated	25	25	25	29	25
Maternal Mortality	0	0	1	7	3
Litters Born (Live)	23	18	20	8	13
Stillborn Litters	0	0	0	2	3
No. Litter Produced[a]	2	7	4	12	6
Mating Efficiency (Live Litters/ No. Mated)	92%	72%	68%	28%**	52%**
No. Pups Born	243	174	178	81	122
No. Pups Stillborn	5	5	16	20	18
Mean Litter Size at Birth ± S.E. (Live Pups)	10.6±.51	9.7±.87	8.9±.87	10.1±1.10	9.4±.78
Mean Birth Weight/ Pup ± S.E. (Live)	1.61±.10	1.59±.13	1.58±.13	1.45±.16*	1.58±.16
Atypical Pups[b]	2	8*	7*	5*	14**

Legend:

These experiments were performed prior to the tenure of the grant and are included for completeness since a substantial amount of data analysis as well as observation of additional characters were performed during the grant period. A manuscript describing these experiments, also written during the tenure of the grant will appear in Teratology 23(1), 1981.

a. Non productive females were not autopsied.

b. Atypical pups, while seen in all groups, had significantly inincreased frequencies of occurrence in experimental groups (* = P<.05, ** = P<.001) compared with untreated. The peculiarities included runting, seen in all groups, mild hemorhaging on the snout, tail, or feet, and possible cases of tail kinks seen in 245T, Trifluralin, and Vehicle treated litters. Six pups from TFL and one each in 245T-H and 235T-L showed little or no movement of the hind limbs, which were crossed in the midline. These pups appeared to have narrow pelvices and were unable to right themselves when turned on their backs. They died within 24 hours, as did several of the runts.

Table All. Vital Statistics Thalidomide/Captan Study

	UNTD	VEH	THAL-L	THAL-H	CAPT
No. Females Treated	28	20	24	20	29
Maternal Mortality	0	0	0	0	1
Litters Born (Live)	25	13	20	14	16
Litters Stillborn	0	0	0	0	0
No. Litter Produced[a]	3	7	4	6	12
Mating Efficiency (Live Litters/ No. Mated)	89%	65%	83%	70%	55%
No. Pups Born	225	119	181	125	145
No. Pups Stillborn	0	2	0	0	2
Mean Litter Size ±S.E. at Birth (Live)	9.0±.65	9.2±.92	9.1±.69	8.9±.78	9.1±.46
Mean Birth Weight ±S.E./Pup (Live)	1.65±.04	1.60±.05	1.63±.04	1.68±.06	1.63±.03
No. Litters Weighed	(23)	(13)	(17)	(8)	(11)

Legend: These experiments were performed prior to the tenure of the grant and are included for completeness. Substantial data analysis was performed on these data during the grant period, as well as observations of additional characters.

a = Non-productive females were not autopsied.

Appendix B. Observations at 18 days post coitus, of fetuses whose dams had been treated with test compounds using the same regimes as for SVAS studies.

Fetal studies were performed with each new compound tested, in order to provide for the post-natal studies a base-level comparison with traditional teratogenic studies. Treatments are described in Table 2 in the report.

Fetal weights were calculated by weighing together all of the live fetuses from a litter and calculating a mean pup weight for each litter. The weight in the tables is a mean of the means, with N being the number of litters. Litter size includes only live fetuses. Frequencies of abnormality and mortality are simple arithmetic means with the number of fetuses or implantations, respectively, as the sample size. Mortality includes both resorption sites and dead fetuses; abnormality is the frequency of fetuses with one or more malformations.

Skeletal parameters were calculated as arithmetic frequencies (N=individuals) or mean litter percents (N=litters) as seemed appropriate.

Table B1. Response at 18 days post coitus of fetuses treated by intubation during days 6-15 of gestation with 1200 mg Maneb/kg/day in 1.0% carboxymethylcellulose

	Untreated	Maneb	t-test
Number of Litters	10	20	-
Mean Dam Weight at 6 DPC (gm)	33.2±0.6	32.4±0.4	NS
Number of Fetuses (Total Live)	100	166	-
Bent Tails	8 (8.0±2.7)	30 (18.1±3.0)	p<.02
Hind Limb Malpositioned	15 (15.0±3.6)	14[a] (8.4±2.2)	NS
Undersized (growth retardation)	5 (5.0±2.2)	14 (8.4±2.2)	NS
Abnormal	0 (0)	10[b] (6.0±1.8)	p<.02
Dead	1	10	-
Resorbed	7	32[c]	-
Mean Litter Size (±S.E.)	10.0±1.2	8.3±0.75	NS
Mean Implantations per Litter (±S.E.)	10.8±1.3	10.4±0.5	NS
Average Weight-Live Fetuses (Mean Litter Weight)	1.26±.02	1.25±.03	NS
% Mortality (±S.E.)	7.5±2.5	20.2±2.8	p<.001

Legend:

a. Includes two dead fetuses

b. Includes a dead fetus with exencephaly, four fetuses with odd head shape, four with abnormal appearance of nasal region, and one individual with spine curvature

c. Includes one completely resorbing litter of ten.

Table B2. Observations at 18 DPC of litters of CD-1 mice from the first series of females treated on days 7, 8, and 9 PC with ip injections of trypan blue.

Treatment	No. of Litters	Normal	Abnormal	Dead	Fetal Weight ($\bar{X}$ S.E.±gm)	Litter Size ($\bar{X}$±S.E.)	%±S.E.		Descrip. of Abnormalities
							Abnormality	Mortality	
UNTD (Untreated)	10	113	1	13	1.28±.04	11.4±1.1	0.9±0.9	10.2±2.7	a
VEH (0.9% NaCl)	10	95	0	20	1.39±.03	9.5±0.8	0	17.4±3.5	
.037TB (3.13mg/kg)	10	83	1	44	1.29±.04	8.4±1.0	1.2±1.2	34.4±4.2	b
.075TB (6.25mg/kg)	10	77	6	48	1.26±.02	8.3±0.8	7.2±2.8	36.6±4.2	c
.15 TB (12.5mg/kg)	10	64	6	62	1.28±.03	7.0±0.6	8.6±3.3	47.0±4.3	d
.3TB (25mg/kg)	10	68	4	51	1.38±.04	7.2±0.7	5.6±2.7	41.5±4.4	e

Comments: Description of Abnormalities: a = one tail kink; b = one short tail; c = 3 tail kinks, 2 tail kinks with short tail, 1 herniated intestines; d = 4 tail kinks, 1 tail kinks with short tail, 1 short tail; e = 3 tail kinks, 1 tail kinks with short tail

In addition to the abnormalities listed, there were in all groups numerous subtle differences among fetuses including tail hooks and waves, as well as small fetuses; since there occurred in UNTD and VEH and were not dose related in the trypan blue groups they were not included.

Table B3. Observations at 18 DPC of litters of CD-1 mice from the first series of experiments, following maternal injection of Acetazolamide.

Treatment	No. of Litters	Number of Fetuses Normal	Number of Fetuses Abnormal	Number of Fetuses Dead	Fetal Weight ($\bar{X}$±S.E. gm)	Litter Size $\bar{X}$±S.E.	%±S.E. Abnormality	%±S.E. Mortality	Description of Abnormalities
UNTD	10	99	0	11	1.34±.03	9.9±1.13	0	10.0±3.4	
VEH-8	11	100	0	21	1.37±.03	9.1±1.16	0	17.4±3.4	
ACZM-L8	10	107	5	21	1.28±.02	11.2±0.55	4.5±2.0	15.8±3.2	a
ACZM-H8	10	87	16	15	1.23±.03	10.3±0.52	15.5±3.6	12.7±3.1	b
VEH-9X3	10	103	4	16	1.25±.04	10.7±0.70	3.7±1.8	13.0±3.0	c
ACZM-L9X3	10	78	10	12	1.32±.06	8.8±1.33	11.4±3.4	12.0±3.2	d
ACZM-H9X3	10	14	74	33	1.07±.04	8.8±1.08	84.1±3.9	27.3±4.1	e

Legend and Notes:

a. 3 cases of exencephaly, exophthalmia and micrognathia, including one with tail kinks; two cases of tail kinks only.

b. 9 cases of exencephaly, exophthalmia and micrognathia, including one with tail kinks; one exencephaly, micrognathia and tail kinks; 4 tail kinks only; one with a short tail.

c. In the VEH-9X3 group there was one fetus with intestinal herniation and 4 with tail kinks.

d. 10 fetuses in three litters (5,4,1) with tail kinks.

e. Among high dose given on three consecutive days, there were 43 cases of Ectrodactyly of the front foot, 40 of them on the right foot only, two on both feet and one with Ectrodactyly on the left and polydactyly on the right. Two other cases of polydactyly occurred on the right front foot as well, one as a solitary malformation, the other in association with tail kinks. Of the 43 cases of ectrodactyly, all but 10 had one toe missing (i.e. had 3 toes present); of those 10, six had 2 toes, and 4 had only one toe. Tail kinks occurred in 56 individuals; 27 cases were associated with Ectrodactyly, and 28 showed this as the only malformation. Five of these were severe enough that the tail was curled, and in 4 of the 5 the tail was short as well. Miscellaneous malformations included 1 fetus with a bent toe, and one with bilateral microphthalmia in addition to ectrodactyly and tail kinks.

Table B4. Observations at 18 DPC of CD-1 mice from the first series of experiments, following maternal injection of 5-Bromodeoxyuridine.

Treatments	No. of Litters	Fetuses			Litter Size ($\bar{X}$±S.E.)	Fetal Weights ($\bar{X}$±S.E. gm)	%±S.E.		Description of Abnormalities
		Normal	Abnormal	Dead			Abnormality	Mortality	
UNTD	10	112	1	11	11.3±0.7	1.36±0.4	0.9±0.9	8.9±2.6	a
VEH-7	10	115	1	10	11.6±1.1	1.29±.03	0.9±0.9	7.9±2.4	b
BUDR-L7	10	100	1	14	10.1±0.9	1.25±.03	1.0±1.0	12.2±3.1	c
BUDR-H7	11	83	28	20	10.1±0.9	1.20±.03	25.2±4.1	15.3±3.1	d
VEH-8	10	103	3	20	10.6±0.4	1.34±.03	2.8±1.6	15.9±3.3	e
BUDR-L8	10	96	2	15	9.8±1.3	1.38±.04	2.0±1.4	13.3±3.2	f
BUDR-H8	11	86	38	16	11.4±1.5	1.24±.05	30.6±4.1	11.4±2.7	g

Legend:

a. One with exencephaly, micrognathia and open eye
b. One with a malformed toe
c. One with curled tail - probably a severe kink
d. 17 cases of exencephaly, micrognathia and exophthalmia one of which also had polydactylism on the right hind foot; another 7 cases of exencephaly and micrognathia only, one case of exophthalmia alone, and one fetus with a tail-kink
e. 3 fetuses, one in each of three litters, had tail kinks
f. 2 fetuses, one in each of two litters, had tail kinks
g. 17 cases of exencephaly, micrognathia, and exophthalmia, three of which also had polydactyly of the hind feet (one on the right foot, two on the left foot); another 4 fetuses with exencephaly and micrognathia only; 13 additional cases of polydactylism of the hind feet (5 right foot, 4 left foot, 4 on both feet; and one fetus with tail kinks only.

Table B5. Observations at 18 DPC of CD-1 mice, following maternal intra-muscular injection of Cortisone Acetate on days 11-14 PC.

Treatments	No. of Litters	Fetuses			Litter Size ($\bar{X}$±S.E.)	Fetal Weights ($\bar{X}$±S.E. gm)	%±S.E.		Description of Abnormalities
		Normal	Abnormal	Dead			Abnormality	Mortality	
UNTD	10	113	2	3	11.5±.8	1.33±.04	1.7±1.2	2.5±1.4	a
Vehicle	10	104	1	5	10.5±1.1	1.40±.05	1.0±1.0	4.6±1.9	b
0.15mg Coristone	10	120	0	5	12.0±.6	1.33±.04	0	4.0±1.8	
0.30mg Cortisone	10	119	3	8	12.2±.7	1.30±.02	2.5±1.4	6.2±2.1	c
0.6mg Cortisone	10	73	39	11	11.2±.6	1.22±.02	34.8±4.5	8.9±2.6	d
1.25mg Cortisone	10	37	74	20	11.0±1.0	1.22±.04	66.7±4.5	15.3±3.2	e

Legend:

a. One cleft palate (CP), one tail kink (note: previous prenatal studies did not include examination for CP, and so no other data are available for spontaneous frequencies of this malformation)
b. One cleft palate
c. One CP, two tail kinks
d. 35 CP, 4 tail kinks
e. One exencephaly with tail kinks, 70 cleft palates of which 5 had tail kinks; two tail kinks.

Table B6. Observations at 18 DPC of litters of CD-1 mice from females treated on days 11-13 PC with ip injections of Diphenylhydantoin

Treatment	No. of Litters	No. of Fetuses			Fetal Weight ($\bar{X}$±S.E. gm)	Litter Size ($\bar{X}$±S.E.)	% S.E.	
		Normal	Abnormal	Dead			Abnormality	Mortality
UNTD (Untreated)	10	104	0	9	1.36±.04	10.4±.7	0	8.0±2.5
VEH (0.05% NaOH)	10	107	0	12	1.31±.04	10.7±.7	0	10.1±2.8
12.5 mg DPH	10	106	0	15	1.22±.06	10.6±.7	0	12.4±3.0
25 mg DPH	10	107	1	13	1.39±.05	10.8±.7	0.93±.93	10.7±2.8
50 mg DPH	10	111	1	10	1.35±.03	11.2±.9	0.89±.89	8.2±2.5

Legend:

The only abnormalities noted were two individuals with cleft palate, one in each of the two highest doses.

Table B7. Ossification of Cervical Centra and Caudal Vertebrae Among CD-1 Mice Examined at 18 Days Post Coitus Following Treatment of Dams as Indicated.

	Number of Individuals											Unossified Cervical Centra			Ossified Caudal Vertebrae	
	Unossified Cervical Centra							Ossfied Caudal Vertebra						$\bar{X}$		
Treatment Group	1	2	3	4	5	6	7	4-6	7-9	10-12	>12	% Affected Litters	Individuals ML%±S.E.	Unossified Per Affected Individual	Six or fewer (%±S.E.)	Average Number (±S.E.)
Bromodeoxyuridine																
Untreated	22	10	16	17	16	17	10	25	53	29	7	40	17.5±10.0	4.9	21.9±3.9	8.5±.2
Water Veh.-Day 7	10	2	7	4	3	2	1	40	71	5	0	30	10.3±5.7	2.4	34.5±4.4	7.0±.1
60mg/kg-Day 7	19	9	15	12	9	9	7	25	65	11	0	50	18.6±8.1	4.2	24.8±4.3	7.5±.2
300mg/kg-Day 7	27	30	42	51	42	41	28	46	56	8	0	100	50.6±9.5	3.5	41.4±4.7	7.0±.2
Water Veh.-Day 8	5	3	6	5	6	7	4	15	59	28	4	40	6.7±3.7	5.1	14.2±3.4	8.6±.2
60mg/kg-Day 8	3	1	3	2	3	3	1	11	67	20	0	50	6.6±2.8	2.3	11.2±3.2	8.2±.2
300mg/kg-Day 8	31	28	42	43	41	33	25	74	41	10	0	82	45.1±11.1	4.8	59.2±4.4	6.6±.2
Acetazolamide																
Untreated	8	2	5	5	4	5	3	39	51	9	0	30	14.2±9.9	3.56	39.4±4.9	7.1±.2
Water Veh.-Day 8	8	2	5	7	4	2	2	29	51	19	1	40	10.7±5.1	2.73	29.0±4.5	7.6±.2
200mg/kg-Day 8	18	8	9	8	7	9	6	55	55	0	0	60	16.3±8.8	3.61	49.1±4.7	6.6±.1
1000mg/kg-Day 8	29	10	19	19	16	15	10	48	50	5	0	80	30.6±7.6	3.58	46.6±4.9	6.7±.2
Water Veh.-Days 9-11	15	4	14	7	8	8	2	57	47	2	0	80	24.8±7.1	2.35	53.3±4.8	6.3±.2
200mg/kg-Days 9-11	15	7	11	13	11	9	7	48	35	4	0	50	19.0±8.4	3.84	54.5±5.3	6.5±.2
1000mg/kg-Days 9-11	34	23	33	29	30	31	19	83	2	0	0	90	56.3 10.9	4.33	94.3±2.5	5.0 .1
Trypan Blue																
Untreated	19	6	11	9	8	8	3	71	41	2	0	70	18.6±6.0	2.78	63.4±4.6	5.4±.1
0.9% NaCl-Vehicle	1	0	2	2	0	2	0	25	59	11	0	20	4.0±3.1	1.75	26.3±4.5	6.2±.2
0.037%	19	4	5	8	6	10	3	47	31	6	0	80	35.7±7.8	2.29	56.0±5.4	5.5±.2
0.075%	40	26	29	33	28	32	17	61	22	0	0	90	57.1±9.3	4.46	73.5±4.8	4.1±.2
0.15%	30	14	19	20	21	22	11	36	33	1	0	90	54.4±8.7	3.70	51.4±6.0	5.4±.2
0.30%	26	10	14	15	16	16	6	31	41	0	0	90	55.6±9.5	2.86	43.1±5.8	5.7±.2

Table B7. Continued

	Number of Individuals											Unossified Cervical Centra			Ossified Caudal Vertebrae	
	Unossified Cervical Centra							Ossified Caudal Vertebra								
Treatment Group	1	2	3	4	5	6	7	4-6	7-9	10-12	>12	% Affected Litters	Individuals ML%±S.E.	$\bar{X}$ Unossified Per Affected Individual	Six or fewer (% S.E.)	Average Number (±S.E.)
Maneb																
Untreated	16	11	14	13	12	13	4	29	68	2	0	90	31.4±10.7	3.6	29.3±4.6	7.1±.1
1200mg/kg	65	49	65	60	58	57	41	78	82	7	0	95	56.0±7.0	4.5	46.7±3.9	6.9±.1
Cortisone																
Untreated	11	9	13	11	11	11	6	20	56	36	3	50	12.3±8.2	4.8	17.4±3.5	8.5±.2
.85% NaCl-Vehicle	4	5	10	7	7	4	4	19	57	28	1	30	9.2±6.6	4.1	18.1±3.8	8.3±.2
0.15mg	3	1	6	6	3	4	1	33	57	29	1	30	8.7±6.0	2.7	27.5±4.1	8.0±.2
0.30mg	4	1	3	3	1	1	1	17	71	31	2	30	3.8±2.4	2.8	13.9±3.1	8.4±.2
0.60mg	18	6	12	10	7	6	3	58	52	2	0	90	20.3±4.7	3.0	51.8±4.7	6.5±.1
1.20mg	25	6	11	8	8	6	4	58	46	6	0	60	22.7±7.7	2.5	52.3±4.7	6.7±.2
Diphenylhydantoin																
Untreated	4	0	2	1	0	0	0	19	66	17	2	40	5.5±7.2	1.4	18.3±3.8	8.1±.2
.05% NaOH Vehicle	7	0	2	2	1	1	0	48	56	3	0	40	6.6±7.9	1.9	44.9±4.8	6.8±.2
12.5 mg	7	0	0	0	0	0	0	51	53	2	0	70	7.0±8.1	1.0	48.1±4.9	6.6±.2
25 mg	6	0	0	0	0	0	0	45	54	9	2	30	5.1±7.0	1.0	41.3±4.7	7.2±.2
50 mg	7	0	0	0	0	1	0	42	60	10	0	40	5.6±7.3	1.1	37.5±4.6	7.2±.2

Table B8. Number of Ribs and Ossified Sternebrae Among CD-1 Mice Examined at 18 Days Post Coitus Following Treatment of Dams as Indicated.

	Number of Individuals						Fourteen Ribs				Ossified Sternebrae			
	Fourteen Ribs			Ossified Sternebrae			ML%±S.E.	%±S.E.			% with			Average Number
Treatment Group	L	R	B	3	4	5	Individuals	L	R	Asymmetry	3	4	5	X±S.E.
Bromodeoxyuridine														
Untreated	3	7	26	0	113	1	31.5±9.8	25.4	29.0	27.8±7.5	0	99.1	.9	4.0±.01
Water Veh-Day 7	13	8	39	0	102	14	48.8±7.8	42.8	40.5	35.0±6.2	0	87.9	12.1	4.1±.03
60mg/kg-Day 7	8	3	25	1	97	2	33.6±7.0	32.7	27.7	30.6±7.7	1.0	96.0	2.0	4.0±.04
300mg/kg-Day 7	10	3	64	2	99	10	69.0±5.0	66.7	60.4	16.9±4.2	1.8	89.2	9.0	4.1±.03
Water Veh-Day 8	8	6	26	1	105	0	38.4±8.0	32.1	30.2	35.0±7.5	.9	99.1	0	4.0±.01
60mg/kg-Day 8	8	6	12	0	96	2	24.5±9.3	20.4	18.4	53.8±9.8	0	98.0	2.0	4.0±.01
300 mg/kg-Day 8	4	6	81	0	116	9	69.2±10.2	68.0	69.6	11.0±3.3	0	92.8	7.2	4.1±.03
Acetazolamide														
Untreated	7	7	23	0	84	15	34.5±9.3	30.3	30.3	37.8±8.0	0	84.9	15.2	4.2±.04
Water Veh-Day 8	3	5	11	0	96	4	20.6±6.6	14.0	16.0	42.1±11.3	0	96.0	4.0	4.0±.02
200mg/kg-Day 8	4	14	79	1	96	15	86.3±4.1	74.1	83.0	18.6±4.0	.9	85.7	13.4	4.1±.03
1000mg/kg-Day 8	1	1	82	0	73	30	79.8±6.4	80.6	80.6	2.4±1.7		70.9	29.1	4.3±.04
Water Veh-Days 9-11	4	4	14	0	102	4	20.8±9.0	16.8	16.8	36.4±10.3	0	95.3	3.7	4.0±.02
200mg/kg-Days 9-11	2	0	11	0	83	5	11.7±4.2	14.8	12.5	15.4±10.0	0	94.3	5.7	4.1±.02
1000mg/kg-Days 9-11	1	1	9	10	76	2	10.0±5.2	11.4	11.4	18.2±11.6	11.4	86.4	2.3	3.9±.04
Trypan Blue														
Untreated	7	5	35	0	112	2	39.8±9.2	36.8	35.1	25.5±6.4	0	98.3	1.8	4.0±.01
0.9% NaCl-Vehicle	6	7	39	0	76	19	53.6±9.9	47.4	48.4	25.0±6.0	0	80.0	20.0	4.2±.04
0.037%	8	6	29	0	77	7	48.7±6.7	44.1	41.7	32.6±7.2	0	91.7	8.3	4.1±.03
0.075%	4	7	30	1	74	8	93.7±5.5	41.0	44.6	26.8±6.9	1.2	89.2	9.6	4.1±.04
0.15%	4	9	36	3	60	7	73.1±9.3	57.1	64.3	26.5±6.3	4.3	85.7	10.0	4.1±.04
0.30%	6	6	35	0	60	12	62.8±8.4	56.9	56.9	25.5±6.4	0	83.3	16.7	4.2±.04
Maneb														
Untreated	8	7	22	1	90	8	36.5±8.5	30.3	29.3	40.5±8.1	1.0	90.9	8.1	4.1±.03
1200 mg/kg	14	24	90	3	156	8	77.3±4.7	62.3	68.3	29.7±4.0	1.8	93.4	4.8	4.0±.02

Table B8. Continued.

	Number of Individuals													
	Fourteen Ribs			Ossified Sternebrae			Fourteen Ribs				Ossified Sternebrae			
							ML%±S.E.	%±S.E.			% with			Average Number
Treatment Group	L	R	B	3	4	5	Individuals	L	R	Asymmetry	3	4	5	$\bar{X}$±S.E.
Cortisone														
Untreated	8	6	28	0	110	5	33.8±7.8	31.3	29.6	33.3±7.3	0	95.7	4.3	4.0±.02
.85% NaCl-Vehicle	6	6	23	0	100	5	31.1±8.5	27.6	27.6	34.3±8.0	0	95.2	4.8	4.1±.02
0.15 mg	4	4	11	0	117	3	16.4±5.5	12.5	12.5	42.1±11.3	0	97.5	2.5	4.0±.01
0.3 mg	12	6	25	0	111	11	35.4±7.5	30.3	25.4	41.9±7.5	0	91.0	9.0	4.1±.03
0.6 mg	1	4	18	1	108	3	20.2±8.6	17.0	19.6	21.7±8.6	.9	96.4	2.7	4.0±.02
1.2 mg	4	5	10	1	108	1	20.5±8.2	12.7	13.6	47.4±11.5	.9	98.2	.9	4.0±.01
Diphenylhydantoin														
Untreated	9	5	32	0	98	6	46.8±10.0	39.4	35.6	30.4±6.8	0	94.2	5.8	4.1±.02
.05% NaOH Vehicle	4	4	17	0	106	1	23.6±8.1	19.6	19.6	32.0±4.5	0	99.1	.9	4.1±.01
12.5 mg	7	3	20	0	106	0	28.7±8.0	25.5	21.7	33.3±4.6	0	100	0	4.0±0
25 mg	13	2	38	0	107	2	48.8±8.4	46.8	36.7	28.3±4.3	0	98.2	1.8	4.0±.01
50 mg	8	5	28	0	105	7	34.9±10.3	32.1	29.5	31.7±4.4	0	93.8	6.3	4.1±.02

Note: For the 14-rib analysis, Asymmetry was calculated as Left only + Right only/Left only + Right only + Both X 100, the L and R columns present total occurrence on each side (L = Left only + Both Sides/Number in sample X 100, etc.)

Table B9. Ossification of Phalanges, Metacarpals, and Metatarsals Among CD-1 Mice Examined at 18 Days Post Coitus, Following Treatment of Dams as Indicated.

	Number of Individuals																Frequency of Incomplete Ossification			
	No. of Ossified Metacarpals					No. of Ossified Metatarsals			Rows of Ossified Phalanges Forelimbs				Rows of Ossified Phalanges Hindlimbs				(%±S.E.) M. Carp.	(%±S.E.) M. Tars.	Phalanges (ML%±S.E.) Forelimb	Phalanges (ML%±S.E.) Hindlimb
Treatment Group	1	2	3	4	5	3	4	5	0	1	2	3	0	1	2	3				
Bromodeoxyuridine																				
Untreated				114				114		1	14	99	1	5	76	32	0	0	12.5±3.7	68.9±9.5
Water Vehicle-Day 7				116				116		1	6	109			95	21	0	0	10.3±8.4	82.0±6.6
60 mg/kg-Day 7			1	99	1		8	93	4	6	16	75	9	5	76	1	1.0±1.0	7.9±2.7	26.3±10.8	90.2±3.1
300 mg/kg-Day 7			1	110			6	105	2	5	22	82	8	9	87	7	0.9±0.9	5.4±2.1	26.9±10.6	94.2±2.7
Water Vehicle-Day				106			3	103	1	3	10	92	3	1	74	28	0	2.8±1.6	13.7±4.8	72.3±7.4
60 mg/kg-Day 8				98				98		3	7	88		1	64	33	0	0	12.7±8.6	70.7±8.1
300 mg/kg-Day 8				125			1	119 5*		4	30	91		10	102	13	0	.80±.80	27.2±11.0	77.7±11.0
Acetazolamide																				
Untreated				99			1	98		3	3	93		2	63	35	0	1.7±1.7	11.7±9.9	66.4±11.4
Water Vehicle-Day 8				100			1	99		1	4	95	1	0	62	37	0	.8±.8	3.1±3.1	65.4±9.8
200 mg/kg-Day 8				112			7	105		1	4	107	1	3	83	25	0	3.9±2.1	4.7±2.1	77.4±5.5
100 mg/kg-Day 8			2	101			10	93			17	86	5	4	76	18	1.9±1.3	7.2±4.8	14.7±10.1	83.1±7.4
Water Vehicle-Days 9-11				107			2	105		2	5	100	1	4	72	30	0	1.7±1.2	9.5±5.7	70.8±10.2
200 mg/kg-Days 9-11				88			3	85			5	83		5	48	35	0	2.8±2.0	4.7±2.6	4.1±14.6
1000 mg/kg-Days 9-11		7	34	47			18	70	1	6	40	41	6	27	54	1	39.0±10.0	31.3±10.2	46.9±11.7	98.8±1.3
Trypan Blue																				
Untreated				114			2	112		1	5	108		1	84	29	0	1.5±1.0	8.6±4.6	71.6±10.1
0.9% NaCl Vehicle				95				95				95			26	69	0	0	0	32.0±10.5
0.037%				84			2	82		1	5	78		5	56	23	0	2.9±2.1	9.8±5.0	77.6±10.1
0.075%				83			7	76		1	6	76			7	76	0	6.8±3.6	7.7±4.8	72.7±10.9
0.15%				70			1	69			9	61	2	4	46	18	0	1.4±1.4	13.0±6.5	73.5±8.9
0.30%				72			5	67			5	67		3	45	24	0	8.9±5.1	12.2±10.0	73.0±13.1

(* = Six metatarsals in each of five individuals, in association with polydactylism occasioned by the treatment)

Table B9. Continued

Treatment Groups	Number of Individuals No. of Ossified Metacarpals 1	2	3	4	5	Metatarsals 3	4	5	Rows of Ossified Phalanges Forelimbs 0	1	2	3	Hindlimbs 0	1	2	3	Frequency of Incomplete Ossification (%±S.E.) M. Carp.	M. Tars.	Phalanges (ML%±S.E.) Forelimb	Hindlimb
Maneb																				
Untreated					99			99		1	39	59		3	93	3	0	0	36.8±9.3	94.9±3.3
1200 mg/kg					168		10	158	3	7	57	101	11	9	142	6	0	5.9±1.8	41.3±6.8	95.5±2.4
Cortisone																				
Untreated					115			115	1	10	4	100	4	10	35	66	0	0	11.6±9.9	39.9±12.8
.85% NaCl Vehicle					105			105		2	7	96	1	4	48	52	0	0	9.2±6.4	47.6±13.0
0.15 mg					120			120		1	7	112		1	46	73	0	0	7.5±4.5	41.5±12.0
0.30 mg					122		1	121		1		121		1	29	93	0	0.8±0.8	0.8±0.8	23.3±11.3
0.60 mg					112		2	110	2		3	107	2	2	37	71	0	1.8±1.3	4.8±1.7	36.2±9.7
1.20 mg					110			110		1	3	106		3	50	57	0	0	3.6±2.8	46.0±9.6
Diphenylhydantoin																				
Untreated					104			104			1	103		2	21	81	0	0	1.4±1.7	25.1±9.3
.05% NaOH Vehicle					107		2	105			3	104		2	43	62	0	2.8±1.6	3.0±1.6	38.6±10.9
12.5 mg					106			106			1	105			60	46	0	0	0.8±0.9	57.1±10.7
25 mg					109			109				109			15	94	0	0	0	12.5±7.6
50 mg					112			112		1	1	110	2	0	40	70	0	0	0	36.3±10.1

Appendix C. Frequencies of Occurrence (%) of Each Skeletal Variant in Each Experimental Series.

The tables present the number of CD-1 mouse skeletons examined in each group (N) and the frequencies of each character. Character numbers correspond to those in Table 3; abbreviations correspond to those in Table 2. Table 2 also describes each treatment.

Table C1. Frequencies of occurrence of each skeletal variant in 2,4,5-T/Trifluralin experiments.

Character Number	UNTD	VEH	245T-L	245T-H	TFL
N=	94	95	93	95	92
1.	0.0	0.0	0.0	0.0	0.0
2.	0.0	0.0	0.0	0.0	0.0
3.	0.0	0.0	0.0	0.0	0.0
4.	0.0	0.0	0.0	0.0	0.0
5.	0.0	0.0	0.0	0.0	0.0
6.	44.7	50.5	52.7	28.4	43.5
7.	1.1	1.1	0.0	5.3	0.0
8.	94.7	94.7	83.9	94.7	93.5
9.	1.1	3.2	2.2	0.0	3.3
10.	40.4	17.9	24.7	23.2	17.4
11.	4.3	18.9	7.5	18.9	17.4
12.	0.0	0.0	0.0	0.0	0.0
13.	0.0	0.0	0.0	0.0	0.0
14.	3.2	8.4	3.2	20.0	6.5
15.	0.0	2.1	1.1	2.1	0.0
16.	23.4	24.2	10.8	50.5	46.7
17.	0.0	0.0	0.0	13.7	1.1
18.	0.0	0.0	0.0	4.2	0.0
19.	6.4	3.2	2.2	5.3	6.5
20.	1.1	3.2	2.2	8.4	4.3
21.	24.5	24.2	30.1	32.6	32.6
22.	75.5	69.5	69.9	71.6	69.6
23.	8.5	10.5	5.4	23.2	15.2
24.	1.1	1.1	1.1	0.0	13.0
25.	8.5	9.5	7.5	4.2	1.1
26.	13.8	30.5	19.4	46.3	31.5
27.	51.1	46.3	34.4	49.5	47.8
28.	0.0	0.0	0.0	0.0	0.0
29.	57.4	48.4	50.5	45.3	63.0
30.	76.6	77.9	72.0	92.6	82.6
31.	0.0	0.0	0.0	0.0	0.0
32.	0.0	0.0	0.0	0.0	0.0
33.	36.2	37.9	44.1	49.5	62.0
34.	0.0	0.0	0.0	0.0	0.0
35.	36.2	22.1	23.7	44.2	27.2
36.	1.1	1.1	0.0	6.3	1.1
37.	2.1	3.2	2.2	9.5	7.6
38.	0.0	1.1	1.1	0.0	1.1
39.	0.0	0.0	0.0	0.0	0.0
40.	0.0	0.0	0.0	0.0	0.0
41.	0.0	0.0	0.0	0.0	0.0
42.	5.3	12.6	9.7	7.4	15.2
43.	89.4	92.6	94.6	92.6	88.0
44.	0.0	0.0	0.0	0.0	0.0

Table Cl. Continued

Character Number	N=	UNTD 94	VEH 95	245T-L 93	245T-H 95	TFL 92
45.		4.3	10.5	8.6	10.5	8.7
46.		1.1	1.1	1.1	0.0	1.1
47.		8.5	7.4	2.2	60.0	6.5
48.		3.2	3.2	2.2	24.2	4.3
49.		3.2	1.1	11.8	48.4	1.1
50.		0.0	0.0	0.0	0.0	2.2
51.		31.9	40.0	15.1	6.3	19.6
52.		6.4	3.2	7.5	22.1	2.2
53.		7.4	22.1	8.6	27.4	2.2
54.		57.4	49.5	59.1	60.0	78.3
55.		1.1	0.0	0.0	1.1	0.0
56.		1.1	1.1	0.0	1.1	0.0
57.		0.0	0.0	1.1	1.1	0.0
58.		0.0	0.0	0.0	0.0	1.1
59.		0.0	0.0	2.2	0.0	2.2
60.		54.3	52.6	59.1	56.8	45.7
61.		1.1	2.1	1.1	1.1	3.3
62.		3.2	0.0	7.5	45.3	2.2
63.		0.0	6.3	2.2	10.5	6.5
64.		10.6	2.1	8.6	2.1	4.3
65.		1.1	0.0	2.2	1.1	0.0
66.		0.0	2.1	0.0	0.0	2.2
67.		75.5	64.2	78.5	66.3	70.7
68.		10.6	13.7	24.7	14.7	27.2
69.		0.0	3.2	2.2	1.1	2.2
70.		22.3	24.2	32.3	30.5	58.7
71.		0.0	0.0	0.0	1.1	0.0
72.		50.0	41.1	47.3	63.2	45.7
73.		71.3	69.5	67.7	69.5	78.3
74.		55.3	45.3	58.1	31.6	60.9
75.		10.6	15.8	14.0	5.3	23.9
76.		0.0	0.0	1.1	0.0	0.0
77.		0.0	1.1	0.0	0.0	0.0
78.		0.0	0.0	3.2	2.1	0.0
79.		23.4	21.1	15.1	40.0	29.3
80.		1.1	0.0	0.0	0.0	1.1
81.		1.1	1.1	0.0	2.1	4.3
82.		3.2	18.9	17.2	3.2	6.5
83.		31.9	28.4	35.5	32.6	33.7
84.		1.1	1.1	2.2	3.2	2.2
85.		0.0	0.0	0.0	0.0	0.0
86.		0.0	0.0	0.0	0.0	0.0
87.		19.1	25.3	36.6	57.9	42.4
88.		1.1	1.1	0.0	0.0	1.1

Table C2. Frequencies of occurrence of each skeletal variant in Thalidomide/Captan experiments.

Character Number	N=	UNTD 92	VEH 91	THAL-L 92	THAL-H 91	CAPTAN 92
1		0.0	0.0	0.0	0.0	0.0
2.		0.0	0.0	0.0	0.0	0.0
3.		0.0	0.0	0.0	0.0	0.0
4.		0.0	0.0	0.0	0.0	0.0
5.		0.0	0.0	0.0	0.0	0.0
6.		28.3	35.2	35.9	39.6	29.3
7.		1.1	0.0	0.0	0.0	0.0
8.		69.6	74.7	78.3	73.6	82.6
9.		6.5	3.3	4.3	6.6	1.1
10.		15.2	13.2	18.5	13.2	19.6
11.		12.0	13.2	15.2	15.4	12.0
12.		0.0	0.0	0.0	0.0	0.0
13.		0.0	0.0	0.0	0.0	0.0
14.		3.3	18.7	6.5	6.6	14.1
15.		0.0	0.0	4.3	1.1	0.0
16.		12.0	39.6	29.3	27.5	18.5
17.		1.1	1.1	3.3	0.0	0.0
18.		3.3	4.4	9.8	12.1	3.3
19.		2.2	6.6	2.2	2.2	5.4
20.		4.3	1.1	3.3	0.0	3.3
21.		13.0	25.3	19.6	20.9	19.6
22.		88.0	84.6	83.7	84.6	77.2
23.		9.8	12.1	10.9	5.5	10.9
24.		0.0	0.0	0.0	0.0	0.0
25.		13.0	11.0	16.3	18.7	16.3
26.		40.2	47.3	45.7	52.7	31.5
27.		34.8	35.2	27.2	42.9	32.6
28.		0.0	0.0	0.0	0.0	0.0
29.		23.9	37.4	32.6	24.2	35.9
30.		64.1	72.5	64.1	64.8	62.0
31.		0.0	0.0	0.0	0.0	0.0
32.		0.0	0.0	0.0	0.0	0.0
33.		15.2	36.3	26.1	27.5	34.8
34.		0.0	0.0	0.0	0.0	0.0
35.		5.4	8.8	9.8	8.8	3.3
36.		3.3	0.0	1.1	7.7	10.9
37.		0.0	1.1	1.1	6.6	6.5
38.		0.0	3.3	0.0	1.1	1.1
39.		0.0	0.0	0.0	0.0	1.1
40.		0.0	1.1	0.0	0.0	0.0
41.		0.0	0.0	0.0	0.0	0.0
42.		4.3	4.4	2.2	1.1	6.5
43.		82.6	70.3	71.7	79.1	73.9
44.		4.3	3.3	1.1	0.0	6.5

Table C2. Continued

Character Number		UNTD	VEH	THAL-L	THAL-H	CAPTAN
	N=	92	91	92	91	92
45.		23.9	8.8	15.2	15.4	14.1
46.		1.1	0.0	0.0	2.2	0.0
47.		10.9	17.6	17.4	24.2	8.7
48.		7.6	17.6	9.8	6.6	4.3
49.		14.1	23.1	14.1	15.4	3.3
50.		0.0	0.0	0.0	0.0	0.0
51.		4.3	6.6	2.2	8.8	10.9
52.		34.8	34.1	44.6	37.4	30.4
53.		3.3	2.2	1.1	12.1	5.4
54.		25.0	30.8	55.4	42.9	38.0
55.		1.1	1.1	0.0	0.0	0.0
56.		1.1	0.0	0.0	0.0	0.0
57.		0.0	0.0	0.0	0.0	0.0
58.		0.0	0.0	0.0	0.0	0.0
59.		0.0	0.0	2.2	1.1	4.3
60.		40.2	45.1	44.6	58.2	30.4
61.		0.0	2.2	2.2	2.2	1.1
62.		1.1	3.3	9.8	9.9	3.3
63.		0.0	3.3	0.0	2.2	0.0
64.		8.7	4.4	6.5	3.3	12.0
65.		0.0	0.0	0.0	0.0	1.1
66.		0.0	1.1	0.0	0.0	0.0
67.		85.9	84.6	88.0	90.1	88.0
68.		16.3	6.6	17.4	22.0	31.5
69.		0.0	3.3	1.1	4.4	3.3
70.		28.3	58.2	30.4	36.3	35.9
71.		0.0	0.0	0.0	0.0	0.0
72.		18.5	19.8	13.0	8.8	22.8
73.		50.0	42.9	65.2	64.8	57.6
74.		56.5	45.1	58.7	51.6	33.7
75.		10.9	5.5	3.3	14.3	16.3
76.		0.0	1.1	0.0	0.0	1.1
77.		0.0	0.0	0.0	0.0	0.0
78.		0.0	4.4	2.2	0.0	1.1
79.		4.3	6.6	3.3	8.8	8.7
80.		0.0	0.0	1.1	0.0	0.0
81.		2.2	0.0	2.2	3.3	1.1
82.		9.8	9.9	25.0	17.6	13.0
83.		1.1	3.3	4.3	0.0	7.6
84.		0.0	0.0	1.1	1.1	0.0
85.		0.0	0.0	0.0	0.0	0.0
86.		1.1	0.0	0.0	0.0	0.0
87.		12.0	13.2	31.5	25.3	14.1
88.		2.2	1.1	3.3	5.5	3.3

Table C3. Frequencies of occurrence of each skeletal variant in Maneb/Decamethrin experiments

Character			Corn Oil		CMC	
		UNTD	VEH	DMT	VEH	MANEB
Number	N=	104	101	102	100	102
1.		0.0	0.0	0.0	0.0	0.0
2.		0.0	0.0	0.0	0.0	0.0
3.		0.0	0.0	0.0	0.0	0.0
4.		0.0	0.0	0.0	0.0	0.0
5.		0.0	0.0	0.0	0.0	0.0
6.		55.8	51.5	49.0	58.0	70.6
7.		0.0	0.0	0.0	0.0	0.0
8.		55.8	59.4	54.9	59.0	53.9
9.		14.4	17.8	19.6	11.0	17.6
10.		27.9	23.8	16.7	22.0	11.8
11.		1.0	0.0	3.9	0.0	3.9
12.		0.0	0.0	0.0	0.0	0.0
13.		0.0	0.0	0.0	0.0	0.0
14.		9.6	22.8	6.9	27.0	18.6
15.		1.0	0.0	0.0	3.0	0.0
16.		37.5	60.4	64.7	47.0	73.5
17.		0.0	0.0	0.0	0.0	2.0
18.		7.7	2.0	3.9	6.0	2.9
19.		0.0	0.0	0.0	0.0	1.0
20.		4.8	7.9	10.8	2.0	2.9
21.		2.9	26.7	30.4	20.0	27.5
22.		79.8	78.2	67.6	65.0	72.5
23.		7.7	7.9	6.9	9.0	6.9
24.		0.0	0.0	0.0	0.0	0.0
25.		6.7	5.0	2.9	7.0	2.0
26.		14.4	23.8	21.6	23.0	15.7
27.		57.7	50.5	54.9	57.0	26.5
28.		0.0	0.0	0.0	0.0	0.0
29.		53.8	52.5	56.9	53.0	58.8
30.		100.0	100.0	100.0	100.0	99.0
31.		0.0	0.0	0.0	0.0	0.0
32.		0.0	1.0	0.0	0.0	0.0
33.		42.3	38.6	53.9	40.0	39.2
34.		0.0	0.0	0.0	0.0	0.0
35.		12.5	5.0	6.9	4.0	5.9
36.		3.8	3.0	3.9	10.0	2.9
37.		1.9	3.0	1.0	1.0	1.0
38.		0.0	0.0	0.0	0.0	0.0
39.		0.0	0.0	0.0	0.0	0.0
40.		0.0	0.0	0.0	0.0	0.0
41.		0.0	0.0	0.0	0.0	0.0
42.		3.8	5.9	4.9	4.0	7.8
43.		91.3	89.1	78.4	93.0	85.3
44.		94.2	89.1	89.2	88.0	92.2

Table C3. Continued

Character			Corn Oil		CMC	
		UNTD	VEH	DMT	VEH	MANEB
Number	N=	104	101	102	100	102
45.		32.7	39.6	37.3	34.0	28.4
46.		0.0	0.0	2.0	1.0	1.0
47.		4.8	5.0	10.8	6.0	8.8
48.		1.9	8.9	2.9	2.0	9.8
49.		1.0	1.0	2.0	0.0	3.9
50.		13.5	5.0	7.8	9.0	11.8
51.		1.0	0.0	0.0	0.0	0.0
52.		93.3	84.2	94.1	78.0	93.1
53.		5.8	8.9	9.8	9.0	0.0
54.		53.8	61.4	52.0	44.0	55.9
55.		0.0	0.0	2.0	1.0	0.0
56.		1.9	0.0	2.9	1.0	0.0
57.		0.0	0.0	1.0	0.0	0.0
58.		0.0	0.0	0.0	0.0	0.0
59.		1.9	0.0	2.0	1.0	3.9
60.		93.3	95.0	96.1	93.0	91.2
61.		1.0	0.0	1.0	1.0	0.0
62.		1.0	2.0	1.0	0.0	10.8
63.		0.0	1.0	2.9	0.0	1.0
64.		0.0	4.0	6.9	0.0	3.9
65.		0.0	1.0	4.9	0.0	2.0
66.		1.0	1.0	0.0	0.0	0.0
67.		99.0	98.0	98.0	96.0	92.2
68.		4.8	3.0	5.9	6.0	22.5
69.		1.0	0.0	1.0	1.0	0.0
70.		37.5	40.6	38.2	26.0	49.0
71.		0.0	0.0	0.0	0.0	0.0
72.		17.3	19.8	27.5	30.0	27.5
73.		91.3	99.0	100.0	95.0	86.3
74.		20.2	12.9	13.7	27.0	6.9
75.		32.7	18.8	15.7	12.0	51.0
76.		0.0	0.0	0.0	0.0	0.0
77.		0.0	0.0	0.0	0.0	0.0
78.		0.0	1.0	2.0	0.0	1.0
79.		9.6	15.8	27.5	19.0	19.6
80.		1.0	0.0	0.0	1.0	0.0
81.		1.0	0.0	3.9	1.0	6.9
82.		51.0	56.4	36.3	62.0	44.1
83.		1.0	2.0	0.0	1.0	2.9
84.		0.0	0.0	1.0	2.0	0.0
85.		0.0	0.0	0.0	0.0	0.0
86.		0.0	2.0	1.0	0.0	0.0
87.		0.0	4.0	9.8	5.0	7.8
88.		4.8	0.0	0.0	2.0	2.9

Table C4. Frequencies of occurrence of each skeletal variant in the first Trypan Blue series.

Character Number	N=	UNTD 100	VEH 100	.037T 96	.075T 86	0.15T 79	0.30T 71
1.		0.0	0.0	0.0	0.0	0.0	0.0
2.		0.0	0.0	1.0	0.0	0.0	0.0
3.		0.0	0.0	0.0	0.0	0.0	0.0
4.		0.0	0.0	0.0	0.0	0.0	0.0
5.		0.0	0.0	0.0	0.0	0.0	0.0
6.		63.0	58.0	66.7	60.5	69.6	57.7
7.		0.0	0.0	0.0	0.0	0.0	0.0
8.		65.0	59.0	63.5	64.0	67.1	80.3
9.		11.0	17.0	8.3	10.5	1.3	4.2
10.		18.0	19.0	27.1	17.4	25.3	21.1
11.		2.0	0.0	1.0	0.0	3.8	0.0
12.		0.0	0.0	0.0	0.0	0.0	0.0
13.		0.0	0.0	0.0	0.0	0.0	0.0
14.		18.0	11.0	38.5	51.2	57.0	66.2
15.		1.0	0.0	1.0	0.0	0.0	4.2
16.		20.0	19.0	36.5	36.0	35.4	38.0
17.		0.0	0.0	1.0	2.3	0.0	0.0
18.		0.0	1.0	1.0	0.0	0.0	1.4
19.		0.0	1.0	0.0	0.0	0.0	0.0
20.		3.0	3.0	4.2	2.3	1.3	2.8
21.		31.0	19.0	30.2	25.6	24.1	18.3
22.		61.0	75.0	70.8	69.8	64.6	70.4
23.		8.0	6.0	7.3	11.6	11.4	8.5
24.		0.0	0.0	0.0	0.0	0.0	0.0
25.		0.0	0.0	0.0	2.3	1.3	5.6
26.		29.0	36.0	34.4	25.6	34.2	42.3
27.		26.0	28.0	37.5	41.9	45.6	25.4
28.		0.0	0.0	0.0	0.0	0.0	1.4
29.		40.0	40.0	33.3	47.7	57.0	43.7
30.		100.0	100.0	99.0	100.0	100.0	100.0
31.		0.0	0.0	0.0	0.0	0.0	0.0
32.		0.0	0.0	0.0	0.0	0.0	0.0
33.		33.0	21.0	17.7	4.7	25.3	9.9
34.		0.0	0.0	0.0	0.0	1.3	0.0
35.		8.0	17.0	7.3	10.5	16.5	18.3
36.		5.0	0.0	2.1	2.3	3.8	5.6
37.		51.0	47.0	39.6	54.7	50.6	57.7
38.		0.0	0.0	0.0	0.0	0.0	0.0
39.		0.0	0.0	0.0	0.0	0.0	0.0
40.		0.0	0.0	0.0	0.0	0.0	0.0
41.		0.0	0.0	0.0	0.0	0.0	0.0
42.		15.0	8.0	10.4	8.1	8.9	14.1
43.		74.0	72.0	87.5	88.4	82.3	91.5
44.		90.0	91.0	92.7	95.3	91.1	76.1

Table C4. Continued

Character Number	UNTD N= 100	VEH 100	.037T 96	.075T 86	0.15T 79	0.30T 71
45.	5.0	8.0	2.1	4.7	8.9	7.0
46.	0.0	0.0	1.0	1.2	1.3	8.5
47.	12.0	8.0	38.5	40.7	57.0	54.9
48.	4.0	5.0	4.2	4.7	17.7	16.9
49.	1.0	1.0	13.5	10.5	16.5	19.7
50.	0.0	4.0	2.1	1.2	0.0	1.4
51.	2.0	2.0	1.0	1.2	1.3	11.3
52.	64.0	71.0	74.0	70.9	58.2	47.9
53.	10.0	6.0	9.4	7.0	13.9	14.1
54.	36.0	27.0	33.3	59.3	51.9	67.6
55.	1.0	0.0	0.0	0.0	1.3	0.0
56.	3.0	3.0	0.0	0.0	1.3	0.0
57.	0.0	0.0	0.0	0.0	1.3	2.8
58.	1.0	0.0	0.0	1.2	0.0	1.4
59.	0.0	0.0	5.2	2.3	3.8	12.7
60.	81.0	93.0	88.5	89.5	82.3	78.9
61.	0.0	0.0	1.0	1.2	0.0	2.8
62.	2.0	2.0	1.0	3.5	3.8	5.6
63.	0.0	1.0	0.0	0.0	0.0	1.4
64.	2.0	5.0	3.1	3.5	2.5	2.8
65.	0.0	0.0	0.0	0.0	0.0	1.4
66.	0.0	0.0	0.0	0.0	0.0	0.0
67.	94.0	88.0	91.7	94.2	98.7	98.6
68.	5.0	1.0	0.0	2.3	3.8	8.5
69.	1.0	4.0	3.1	5.8	3.8	7.0
70.	43.0	39.0	52.1	47.7	44.3	43.7
71.	0.0	0.0	1.0	1.2	1.3	5.6
72.	20.0	25.0	22.9	22.1	30.4	12.7
73.	49.0	66.0	51.0	55.8	34.2	63.4
74.	13.0	26.0	32.3	23.3	16.5	33.8
75.	27.0	21.0	37.5	52.3	54.4	76.1
76.	0.0	0.0	2.1	0.0	0.0	1.4
77.	0.0	0.0	2.1	2.3	1.3	4.2
78.	2.0	0.0	5.2	9.3	8.9	11.3
79.	12.0	12.0	15.6	11.6	10.1	16.9
80.	0.0	1.0	2.1	1.2	0.0	2.8
81.	8.0	8.0	11.5	16.3	15.2	26.8
82.	72.0	77.0	67.7	66.3	70.9	59.2
83.	1.0	1.0	0.0	0.0	0.0	2.8
84.	0.0	0.0	0.0	0.0	0.0	0.0
85.	0.0	0.0	0.0	0.0	0.0	0.0
86.	1.0	1.0	6.2	3.5	7.6	16.9
87.	4.0	9.0	8.3	9.3	13.9	21.1
88.	5.0	9.0	13.5	14.0	20.3	33.8

Table C5. Frequencies of occurrence of skeletal variant in the second Trypan Blue series.

Character		UNTD	VEH	.037T	.075T	.015T	0.30T
Number	N=	100	97	75	75	65	100
1.		0.0	0.0	0.0	0.0	0.0	0.0
2.		0.0	0.0	0.0	0.0	0.0	0.0
3.		0.0	0.0	0.0	0.0	0.0	0.0
4.		0.0	0.0	0.0	0.0	0.0	0.0
5.		0.0	0.0	0.0	0.0	0.0	0.0
6.		37.0	38.1	45.3	46.7	41.5	41.0
7.		0.0	0.0	0.0	0.0	0.0	0.0
8.		69.0	66.0	84.0	64.0	78.5	72.0
9.		9.0	8.2	1.3	6.7	3.1	1.0
10.		28.0	9.3	13.3	10.7	6.2	6.0
11.		1.0	2.1	1.3	5.3	9.2	6.0
12.		0.0	0.0	0.0	0.0	0.0	0.0
13.		0.0	0.0	0.0	0.0	0.0	0.0
14.		11.0	11.3	49.3	57.3	76.9	68.0
15.		0.0	1.0	5.3	2.7	1.5	4.0
16.		48.0	67.0	69.3	65.3	60.0	68.0
17.		0.0	0.0	0.0	0.0	0.0	0.0
18.		0.0	0.0	1.3	0.0	0.0	0.0
19.		0.0	0.0	0.0	0.0	0.0	0.0
20.		6.0	2.1	5.3	5.3	4.6	9.0
21.		25.0	24.7	10.7	10.7	13.8	14.0
22.		49.0	53.6	77.3	54.7	61.5	58.0
23.		13.0	11.3	2.7	13.3	13.8	12.0
24.		0.0	0.0	0.0	0.0	0.0	0.0
25.		1.0	1.0	2.7	5.3	4.6	1.0
26.		23.0	16.5	21.3	18.7	21.5	26.0
27.		40.0	38.1	53.3	52.0	53.8	51.0
28.		0.0	0.0	0.0	0.0	0.0	0.0
29.		41.0	41.2	36.0	41.3	38.5	46.0
30.		100.0	97.9	97.3	98.7	100.0	98.0
31.		0.0	0.0	0.0	0.0	0.0	0.0
32.		0.0	0.0	0.0	1.3	0.0	0.0
33.		31.0	23.7	17.3	20.0	21.5	13.0
34.		0.0	0.0	0.0	0.0	0.0	0.0
35.		21.0	17.5	22.7	17.3	18.5	18.0
36.		6.0	5.2	4.0	6.7	7.7	2.0
37.		23.0	24.7	22.7	34.7	30.8	39.0
38.		0.0	0.0	0.0	0.0	0.0	0.0
39.		0.0	0.0	0.0	0.0	0.0	0.0
40.		0.0	0.0	1.3	0.0	0.0	0.0
41.		0.0	0.0	0.0	0.0	0.0	0.0
42.		9.0	13.4	12.0	12.0	3.1	10.0
43.		83.0	71.1	82.7	81.3	89.2	86.0
44.		49.0	59.8	30.7	52.0	52.3	52.0

Table C5. Continued

Character		UNTD	VEH	.037T	.075T	.015T	0.30T
Number	N=	100	97	75	75	65	100
45.		16.0	5.2	8.0	4.0	6.2	9.0
46.		0.0	0.0	1.3	2.7	4.6	5.0
47.		9.0	13.4	29.3	48.0	55.4	40.0
48.		11.0	1.0	2.7	1.3	12.3	11.0
49.		3.0	8.2	6.7	14.7	16.9	11.0
50.		2.0	1.0	2.7	4.0	0.0	6.0
51.		0.0	0.0	0.0	0.0	0.0	1.0
52.		82.0	75.3	68.0	66.7	69.2	68.0
53.		8.0	4.1	12.0	8.0	7.7	16.0
54.		26.0	37.1	48.0	70.7	56.9	67.0
55.		1.0	0.0	0.0	0.0	0.0	0.0
56.		2.0	0.0	0.0	0.0	0.0	0.0
57.		0.0	0.0	0.0	0.0	0.0	0.0
58.		0.0	0.0	0.0	0.0	0.0	0.0
59.		0.0	1.0	4.0	5.3	9.2	8.0
60.		51.0	35.1	45.3	48.0	35.4	44.0
61.		1.0	0.0	1.3	1.3	1.5	0.0
62.		2.0	2.1	4.0	2.7	0.0	1.0
63.		1.0	2.1	1.3	0.0	1.5	0.0
64.		11.0	7.2	16.0	17.3	4.6	1.0
65.		0.0	0.0	0.0	0.0	0.0	0.0
66.		0.0	0.0	0.0	0.0	0.0	0.0
67.		99.0	75.3	98.7	81.3	86.2	89.0
68.		1.0	1.0	5.3	0.0	3.1	1.0
69.		0.0	2.1	2.7	5.3	6.2	9.0
70.		68.0	52.6	68.0	56.0	56.9	58.0
71.		0.0	0.0	1.3	1.3	1.5	5.0
72.		14.0	19.6	28.0	33.3	46.2	50.0
73.		27.0	38.1	29.3	37.3	26.2	40.0
74.		15.0	24.7	21.3	9.3	13.8	15.0
75.		21.0	1.0	33.3	64.0	64.6	72.0
76.		0.0	1.0	0.0	0.0	0.0	0.0
77.		2.0	1.0	1.3	2.7	3.1	5.0
78.		0.0	0.0	5.3	8.0	4.6	9.0
79.		0.0	3.1	12.0	18.7	9.2	17.0
80.		1.0	0.0	0.0	0.0	1.5	4.0
81.		1.0	1.0	6.7	10.7	12.3	17.0
82.		11.0	5.2	8.0	17.3	6.2	10.0
83.		0.0	0.0	0.0	0.0	0.0	1.0
84.		1.0	0.0	0.0	0.0	0.0	0.0
85.		0.0	0.0	0.0	0.0	0.0	0.0
86.		1.0	0.0	4.0	6.7	7.7	9.0
87.		2.0	0.0	9.3	10.7	15.4	15.0
88.		4.0	2.1	9.3	16.0	16.9	24.0

Table C6. Frequencies of occurrence of each skeletal variant in the first Acetazolamide series.

Character Number	UNTD	Day 8 treatments			Days 9,10,11 treatments		
		VEH	ACZM-L	ACZM-H	VEH	ACZM-L	ACZM-H
N=	98	97	99	97	100	88	100
1.	0.0	0.0	0.0	1.0	0.0	0.0	0.0
2.	0.0	0.0	0.0	1.0	0.0	0.0	0.0
3.	1.0	0.0	0.0	0.0	0.0	0.0	0.0
4.	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5.	0.0	0.0	0.0	0.0	0.0	1.1	0.0
6.	31.6	25.8	19.2	25.8	31.0	14.8	19.0
7.	0.0	0.0	0.0	0.0	1.0	0.0	0.0
8.	76.5	78.4	68.7	67.0	66.0	73.9	68.0
9.	1.0	2.1	7.1	4.1	0.0	2.3	3.0
10.	23.5	29.9	20.2	19.6	21.0	14.8	14.0
11.	1.0	2.1	1.0	6.2	5.0	1.1	2.0
12.	0.0	0.0	0.0	0.0	0.0	0.0	0.0
13.	0.0	0.0	0.0	0.0	0.0	0.0	0.0
14.	9.2	9.3	10.1	18.6	5.0	6.8	10.0
15.	0.0	1.0	1.0	1.0	1.0	0.0	2.0
16.	44.9	36.1	61.6	72.2	34.0	60.2	54.0
17.	0.0	0.0	0.0	1.0	0.0	1.1	0.0
18.	1.0	1.0	0.0	0.0	0.0	0.0	0.0
19.	0.0	0.0	0.0	0.0	1.0	0.0	1.0
20.	6.1	2.1	22.2	56.7	5.0	4.5	5.0
21.	27.6	17.5	15.2	16.5	17.0	18.2	9.0
22.	71.4	58.8	59.6	64.9	47.0	48.9	46.0
23.	3.1	8.2	5.1	11.3	12.0	11.4	10.0
24.	0.0	0.0	0.0	0.0	0.0	0.0	0.0
25.	0.0	0.0	0.0	1.0	0.0	0.0	0.0
26.	25.5	19.6	31.3	39.2	20.0	21.6	40.0
27.	32.7	40.2	54.5	77.3	37.0	30.7	35.0
28.	1.0	0.0	0.0	1.0	0.0	0.0	0.0
29.	58.2	57.7	47.5	42.3	34.0	42.0	50.0
30.	100.0	100.0	99.0	100.0	99.0	100.0	97.0
31.	0.0	0.0	0.0	0.0	0.0	0.0	0.0
32.	0.0	0.0	0.0	0.0	0.0	0.0	0.0
33.	25.5	22.7	28.3	29.9	41.0	26.1	10.0
34.	0.0	0.0	0.0	0.0	0.0	0.0	0.0
35.	3.1	10.3	8.1	5.2	11.0	6.8	10.0
36.	3.1	2.1	6.1	7.2	1.0	1.1	1.0
37.	37.8	48.5	44.4	47.4	43.0	34.1	31.0
38.	0.0	0.0	0.0	0.0	0.0	0.0	0.0
39.	0.0	0.0	0.0	0.0	0.0	0.0	0.0
40.	0.0	0.0	0.0	1.0	0.0	0.0	0.0
41.	0.0	0.0	0.0	0.0	0.0	0.0	0.0
42.	7.1	12.4	14.1	8.2	23.0	29.5	9.0
43.	75.5	79.4	77.8	66.0	79.0	77.3	82.0
44.	81.6	74.2	78.8	66.0	72.0	69.3	77.0

Table C6. Continued

			Day 8 treatments			Days 9,10,11 treatments		
Character		UNTD	VEH	ACZM-L	ACZM-H	VEH	ACZM-L	ACZM-H
Number	N=	98	97	99	97	100	88	100
45.		20.4	27.8	27.3	28.9	36.0	38.6	54.0
46.		0.0	0.0	0.0	0.0	0.0	0.0	0.0
47.		11.2	7.2	4.0	15.5	8.0	14.8	26.0
48.		1.0	3.1	2.0	0.0	2.0	2.3	6.0
49.		1.0	12.4	4.0	2.1	5.0	0.0	1.0
50.		6.1	5.2	4.0	4.1	0.0	4.5	11.0
51.		1.0	4.1	5.1	1.0	3.0	5.7	2.0
52.		90.8	76.3	82.8	80.4	78.0	70.5	59.0
53.		10.2	7.2	5.1	7.2	13.0	6.8	2.0
54.		45.9	57.7	35.4	26.8	22.0	38.6	47.0
55.		0.0	0.0	0.0	0.0	0.0	0.0	1.0
56.		1.0	0.0	0.0	1.0	0.0	0.0	0.0
57.		0.0	0.0	0.0	2.1	0.0	0.0	0.0
58.		0.0	0.0	0.0	0.0	0.0	0.0	1.0
59.		2.0	1.0	2.0	4.1	0.0	1.1	8.0
60.		79.6	90.7	79.8	82.5	79.0	88.6	78.0
61.		1.0	0.0	0.0	0.0	0.0	0.0	0.0
62.		2.0	3.1	3.0	3.1	3.0	0.0	1.0
63.		0.0	0.0	0.0	0.0	0.0	0.0	0.0
64.		7.1	8.2	4.0	5.2	3.0	12.5	5.0
65.		0.0	0.0	0.0	1.0	0.0	0.0	0.0
66.		0.0	0.0	0.0	0.0	0.0	0.0	0.0
67.		83.7	82.5	84.8	85.6	89.0	94.3	88.0
68.		2.0	1.0	2.0	11.3	3.0	5.7	9.0
69.		2.0	0.0	0.0	2.1	5.0	1.1	6.0
70.		24.5	25.8	87.9	92.8	31.0	12.5	8.0
71.		0.0	0.0	1.0	3.1	0.0	0.0	3.0
72.		16.3	13.4	20.2	10.3	17.0	14.8	7.0
73.		66.3	52.6	50.5	49.5	58.0	78.4	95.0
74.		32.7	38.1	33.3	26.8	19.0	25.0	7.0
75.		5.1	20.6	14.1	21.6	14.0	4.5	5.0
76.		0.0	0.0	0.0	0.0	0.0	1.1	2.0
77.		0.0	0.0	0.0	8.2	1.0	6.8	26.0
78.		0.0	0.0	3.0	21.6	0.0	1.1	1.0
79.		3.1	2.1	11.1	14.4	6.0	26.1	35.0
80.		0.0	0.0	1.0	4.1	0.0	3.4	12.0
81.		1.0	2.1	8.1	11.3	6.0	25.0	37.0
82.		11.2	11.3	5.1	10.3	3.0	4.5	6.0
83.		2.0	0.0	1.0	1.0	0.0	0.0	1.0
84.		0.0	0.0	0.0	0.0	0.0	0.0	0.0
85.		0.0	0.0	0.0	0.0	0.0	0.0	0.0
86.		0.0	0.0	0.0	3.1	0.0	0.0	0.0
87.		2.0	5.2	6.1	3.1	7.0	10.2	33.0
88.		5.1	3.1	3.0	4.1	5.0	37.5	85.0

Table C7. Frequencies of occurrence of each skeletal variant in the second Acetazolamide series.

			Day 8 treatments			Days 9,10,11 treatments		
Character		UNTD	VEH	ACZM-L	ACZM-H	VEH	ACZM-L	ACZM-H
Number	N=	100	100	89	98	83	99	80
1.		0.0	0.0	1.1	2.0	0.0	0.0	0.0
2.		0.0	0.0	0.0	0.0	0.0	0.0	0.0
3.		0.0	3.0	0.0	2.0	0.0	0.0	1.2
4.		0.0	0.0	0.0	0.0	0.0	0.0	0.0
5.		0.0	0.0	0.0	1.0	0.0	0.0	0.0
6.		37.0	33.0	30.3	30.6	31.3	20.2	16.3
7.		0.0	0.0	0.0	0.0	0.0	0.0	0.0
8.		69.0	60.0	50.6	77.6	59.0	66.7	62.5
9.		9.0	4.0	13.5	2.0	3.6	12.1	8.7
10.		28.0	23.0	16.9	11.2	25.3	20.2	27.5
11.		1.0	11.0	3.4	7.1	2.4	1.0	2.5
12.		0.0	0.0	0.0	0.0	0.0	0.0	0.0
13.		0.0	0.0	0.0	0.0	0.0	0.0	0.0
14.		11.0	17.0	28.1	42.9	18.1	10.1	18.7
15.		0.0	0.0	1.1	2.0	1.2	0.0	1.2
16.		48.0	47.0	74.2	91.8	62.7	70.7	95.0
17.		0.0	0.0	2.2	3.1	1.2	1.0	0.0
18.		0.0	2.0	0.0	0.0	0.0	0.0	0.0
19.		0.0	0.0	1.1	0.0	0.0	0.0	0.0
20.		6.0	3.0	41.6	61.2	8.4	4.0	6.2
21.		25.0	15.0	15.7	22.4	15.7	16.2	8.7
22.		49.0	58.0	56.2	65.3	48.2	45.5	45.0
23.		13.0	9.0	10.1	10.2	16.9	14.1	15.0
24.		0.0	0.0	0.0	0.0	0.0	0.0	0.0
25.		1.0	0.0	1.1	0.0	1.2	0.0	1.2
26.		23.0	21.0	40.4	51.0	25.3	14.1	40.0
27.		40.0	41.0	71.9	81.6	27.7	25.3	40.0
28.		0.0	0.0	0.0	0.0	0.0	0.0	0.0
29.		41.0	38.0	33.7	46.9	34.9	46.5	36.3
30.		100.0	100.0	100.0	86.7	100.0	99.0	100.0
31.		0.0	0.0	0.0	0.0	0.0	0.0	0.0
32.		0.0	0.0	0.0	0.0	0.0	0.0	0.0
33.		31.0	40.0	24.7	22.4	37.3	31.3	12.5
34.		0.0	0.0	0.0	0.0	0.0	0.0	0.0
35.		21.0	11.0	14.6	16.3	9.6	13.1	15.0
36.		6.0	7.0	6.7	12.2	2.4	4.0	10.0
37.		23.0	21.0	13.5	25.5	21.7	13.1	11.3
38.		0.0	0.0	0.0	0.0	0.0	0.0	0.0
39.		0.0	0.0	0.0	0.0	0.0	0.0	0.0
40.		0.0	0.0	0.0	0.0	0.0	0.0	0.0
41.		0.0	0.0	0.0	0.0	0.0	0.0	0.0
42.		9.0	28.0	10.1	15.3	7.2	7.1	20.0
43.		83.0	81.0	71.9	72.4	73.5	87.9	85.0
44.		49.0	48.0	33.7	35.7	48.2	45.5	53.8

Table C7. Continued

Character Number	N=	UNTD	Day 8 treatments VEH	Day 8 treatments ACZM-L	Day 8 treatments ACZM-H	Days 9,10,11 treatments VEH	Days 9,10,11 treatments ACZM-L	Days 9,10,11 treatments ACZM-H
		100	100	89	98	83	99	80
45.		16.0	19.0	12.4	18.4	26.5	47.5	62.5
46.		0.0	0.0	0.0	1.0	1.2	0.0	0.0
47.		9.0	9.0	7.9	7.1	2.4	9.1	20.0
48.		11.0	9.0	10.1	2.0	2.4	2.0	2.5
49.		3.0	3.0	1.1	2.0	1.2	3.0	1.2
50.		2.0	2.0	5.6	9.2	4.8	4.0	7.5
51.		0.0	3.0	3.4	1.0	3.6	1.0	0.0
52.		82.0	84.0	77.5	68.4	77.1	58.6	60.0
53.		8.0	7.0	1.1	3.1	4.8	1.0	1.2
54.		26.0	35.0	51.7	36.7	39.8	51.5	52.5
55.		1.0	0.0	1.1	0.0	0.0	0.0	5.0
56.		2.0	1.0	1.1	2.0	0.0	1.0	0.0
57.		0.0	0.0	0.0	1.0	0.0	0.0	0.0
58.		0.0	0.0	0.0	0.0	0.0	0.0	1.2
59.		0.0	2.0	0.0	10.2	0.0	2.0	3.8
60.		51.0	41.0	39.3	50.0	61.4	59.6	58.7
61.		1.0	2.0	1.1	4.1	1.2	0.0	1.2
62.		2.0	3.0	3.4	2.0	0.0	0.0	0.0
63.		1.0	0.0	3.4	1.0	0.0	0.0	0.0
64.		11.0	15.0	19.1	20.4	13.3	17.2	16.3
65.		0.0	0.0	1.1	0.0	0.0	0.0	0.0
66.		0.0	3.0	2.2	0.0	0.0	0.0	0.0
67.		99.0	80.0	82.0	84.7	83.1	79.8	93.7
68.		1.0	3.0	4.5	10.2	4.8	3.0	27.5
69.		0.0	1.0	1.1	4.1	1.2	1.0	1.2
70.		68.0	67.0	97.8	100.0	45.8	21.2	13.8
71.		0.0	0.0	0.0	0.0	0.0	0.0	3.8
72.		14.0	19.0	22.5	27.6	22.9	31.3	35.0
73.		27.0	26.0	25.8	19.4	27.7	47.5	66.3
74.		15.0	18.0	19.1	29.6	7.2	10.1	0.0
75.		21.0	17.0	7.9	24.5	13.3	11.1	10.0
76.		0.0	0.0	0.0	0.0	0.0	0.0	1.2
77.		2.0	0.0	0.0	21.4	1.2	8.1	30.0
78.		0.0	1.0	18.0	40.8	0.0	2.0	3.8
79.		0.0	1.0	4.5	6.1	9.6	10.1	23.8
80.		1.0	0.0	0.0	2.0	0.0	1.0	8.7
81.		1.0	2.0	4.5	2.0	3.6	5.1	20.0
82.		11.0	4.0	16.9	21.4	4.8	3.0	7.5
83.		0.0	0.0	1.1	0.0	1.2	0.0	13.8
84.		1.0	1.0	0.0	0.0	0.0	1.0	2.5
85.		0.0	0.0	0.0	0.0	0.0	0.0	0.0
86.		1.0	0.0	0.0	1.0	0.0	0.0	0.0
87.		2.0	1.0	7.9	2.0	1.2	5.1	12.5
88.		4.0	5.0	11.2	5.1	3.6	24.2	93.7

Table C8. Frequencies of occurrence of each skeletal variant in the first Bromodeoxyuridine series.

Character Number		UNTD	VEH-7	BUDR-L7	BUDR-H7	VEH-8	BUDR-L8	BUDR-H8
	N=	98	97	98	100	98	99	99
1.		0.0	0.0	0.0	1.0	0.0	0.0	0.0
2.		0.0	0.0	0.0	0.0	0.0	0.0	0.0
3.		0.0	0.0	0.0	0.0	0.0	0.0	0.0
4.		0.0	0.0	0.0	0.0	0.0	0.0	0.0
5.		0.0	0.0	0.0	1.0	0.0	0.0	0.0
6.		28.6	28.9	20.4	35.0	16.3	24.2	21.2
7.		0.0	0.0	0.0	0.0	0.0	0.0	0.0
8.		80.6	84.5	78.6	84.0	84.7	83.8	82.8
9.		1.0	1.0	3.1	2.0	3.1	3.0	3.0
10.		15.3	11.3	7.1	12.0	15.3	14.1	6.1
11.		3.1	3.1	5.1	6.0	2.0	4.0	4.0
12.		0.0	0.0	0.0	0.0	0.0	0.0	0.0
13.		0.0	0.0	0.0	0.0	0.0	0.0	0.0
14.		9.2	15.5	8.2	40.0	12.2	11.1	33.3
15.		0.0	1.0	0.0	1.0	0.0	0.0	0.0
16.		73.5	52.6	42.9	79.0	65.3	77.8	80.8
17.		0.0	0.0	0.0	1.0	0.0	0.0	0.0
18.		0.0	0.0	1.0	0.0	1.0	2.0	2.0
19.		0.0	0.0	0.0	1.0	0.0	0.0	0.0
20.		2.0	2.1	0.0	22.0	2.0	4.0	2.0
21.		17.3	15.5	-10.2	22.0	18.4	13.1	19.2
22.		61.2	55.7	67.3	68.0	67.3	68.7	65.7
23.		6.1	10.3	5.1	5.0	4.1	6.1	7.1
24.		0.0	0.0	0.0	0.0	0.0	0.0	0.0
25.		1.0	1.0	1.0	0.0	1.0	1.0	3.0
26.		23.5	33.0	40.8	70.0	39.8	50.5	56.6
27.		69.4	67.0	54.1	72.0	62.2	40.4	66.7
28.		0.0	0.0	0.0	0.0	0.0	0.0	0.0
29.		45.9	44.3	39.8	46.0	49.0	45.5	39.4
30.		91.8	70.1	83.7	94.0	76.5	82.8	87.9
31.		0.0	0.0	0.0	0.0	0.0	0.0	0.0
32.		0.0	0.0	0.0	0.0	0.0	0.0	0.0
33.		39.8	38.1	43.9	42.0	39.8	43.4	43.4
34.		0.0	0.0	0.0	0.0	0.0	0.0	0.0
35.		8.2	12.4	15.3	11.0	9.2	9.1	6.1
36.		0.0	2.1	4.1	2.0	3.1	3.0	7.1
37.		58.2	40.2	55.1	35.0	35.7	36.4	33.3
38.		0.0	0.0	0.0	0.0	0.0	0.0	0.0
39.		0.0	0.0	0.0	0.0	0.0	0.0	0.0
40.		0.0	0.0	0.0	0.0	0.0	0.0	0.0
41.		0.0	0.0	0.0	0.0	0.0	0.0	0.0
42.		6.1	9.3	15.3	9.0	4.1	2.0	1.0
43.		75.5	67.0	75.5	69.0	69.4	76.8	78.8
44.		46.9	47.4	48.0	54.0	45.9	44.4	53.5

Table C8. Continued

Character Number	N=	UNTD 98	VEH-7 97	BUDR-L7 98	BUDR-H7 100	VEH-8 98	BUDR-L8 99	BUDR-H8 99
45.		10.2	18.6	9.2	21.0	19.4	13.1	12.1
46.		0.0	0.0	1.0	2.0	0.0	0.0	0.0
47.		4.1	15.5	12.2	19.0	19.4	15.2	8.1
48.		10.2	5.2	13.3	18.0	3.1	5.1	8.1
49.		0.0	7.2	1.0	2.0	6.1	7.1	12.1
50.		1.0	0.0	1.0	3.0	5.1	1.0	2.0
51.		0.0	0.0	0.0	0.0	0.0	0.0	1.0
52.		78.6	82.5	77.6	76.0	85.7	74.7	76.8
53.		22.4	12.4	15.3	10.0	11.2	10.1	12.1
54.		42.9	37.1	40.8	30.0	38.8	38.4	43.4
55.		1.0	0.0	1.0	0.0	2.0	0.0	0.0
56.		0.0	0.0	1.0	1.0	1.0	3.0	4.0
57.		0.0	0.0	0.0	3.0	1.0	0.0	0.0
58.		0.0	0.0	0.0	1.0	0.0	0.0	0.0
59.		0.0	0.0	0.0	19.0	0.0	1.0	3.0
60.		61.2	73.2	67.3	67.0	73.5	74.7	59.6
61.		1.0	0.0	0.0	2.0	0.0	0.0	2.0
62.		12.2	2.1	6.1	9.0	5.1	16.2	7.1
63.		0.0	1.0	1.0	4.0	0.0	3.0	1.0
64.		4.1	3.1	1.0	2.0	3.1	3.0	1.0
65.		0.0	0.0	1.0	1.0	0.0	3.0	1.0
66.		0.0	1.0	0.0	1.0	0.0	1.0	0.0
67.		74.5	88.7	89.8	83.0	84.7	72.7	83.8
68.		6.1	3.1	4.1	18.0	5.1	7.1	7.1
69.		4.1	7.2	1.0	10.0	6.1	10.1	13.1
70.		36.7	20.6	36.7	81.0	28.6	41.4	83.8
71.		0.0	0.0	0.0	4.0	0.0	0.0	8.1
72.		27.6	35.1	27.6	33.0	24.5	42.4	29.3
73.		42.9	52.6	50.0	46.0	51.0	27.3	22.2
74.		45.9	35.1	55.1	26.0	41.8	34.3	25.3
75.		6.1	4.1	6.1	5.0	5.1	11.1	16.2
76.		0.0	3.1	0.0	2.0	1.0	1.0	0.0
77.		0.0	0.0	0.0	2.0	0.0	1.0	0.0
78.		0.0	0.0	0.0	5.0	0.0	1.0	4.0
79.		2.0	0.0	0.0	3.0	4.1	1.0	2.0
80.		0.0	0.0	0.0	0.0	0.0	0.0	0.0
81.		0.0	0.0	0.0	2.0	1.0	1.0	1.0
82.		19.4	20.6	22.4	6.0	12.2	16.2	3.0
83.		0.0	1.0	0.0	0.0	0.0	0.0	0.0
84.		0.0	0.0	0.0	1.0	0.0	0.0	0.0
85.		0.0	0.0	0.0	0.0	0.0	0.0	0.0
86.		0.0	0.0	2.0	0.0	2.0	1.0	1.0
87.		1.0	4.1	2.0	0.0	5.1	3.0	1.0
88.		3.1	2.1	1.0	8.0	5.1	2.0	1.0

Table C9. Frequencies of occurrence of each skeletal variant in the second Bromodeoxyuridine series.

Character Number	N=	UNTD 97	VEH-7 99	BUDR-L7 97	BUDR-H7 87	VEH-8 96	BUDR-L8 98	BUDR-H8 100
1.		0.0	0.0	0.0	1.1	0.0	0.0	0.0
2.		0.0	0.0	0.0	1.1	0.0	0.0	0.0
3.		0.0	1.0	0.0	0.0	1.0	0.0	2.0
4.		0.0	0.0	0.0	0.0	0.0	0.0	0.0
5.		0.0	0.0	0.0	0.0	0.0	0.0	0.0
6.		33.0	25.3	30.9	26.4	35.4	17.3	20.0
7.		0.0	0.0	0.0	0.0	0.0	0.0	0.0
8.		66.0	67.7	70.1	64.4	66.7	67.3	72.0
9.		0.0	1.0	3.1	0.0	1.0	5.1	2.0
10.		14.4	16.2	14.4	16.1	20.8	14.3	18.0
11.		1.0	0.0	0.0	1.1	1.0	0.0	0.0
12.		0.0	0.0	0.0	0.0	0.0	0.0	0.0
13.		0.0	0.0	0.0	0.0	0.0	0.0	0.0
14.		9.3	5.1	14.4	34.5	8.3	8.2	21.0
15.		1.0	0.0	3.1	1.1	0.0	0.0	1.0
16.		30.9	33.3	33.0	36.8	22.9	27.6	27.0
17.		0.0	0.0	0.0	0.0	0.0	0.0	0.0
18.		1.0	0.0	2.1	0.0	2.1	0.0	0.0
19.		0.0	0.0	0.0	0.0	0.0	0.0	0.0
20.		4.1	0.0	9.3	5.7	1.0	3.1	7.0
21.		22.7	21.2	25.8	31.0	24.0	17.3	28.0
22.		55.7	53.5	52.6	49.4	60.4	48.0	48.0
23.		2.1	2.0	0.0	0.0	2.1	2.0	2.0
24.		0.0	0.0	0.0	0.0	0.0	0.0	0.0
25.		0.0	0.0	0.0	0.0	0.0	0.0	0.0
26.		24.7	27.3	29.9	50.6	28.1	28.6	66.0
27.		33.0	29.3	32.0	37.9	32.3	30.6	33.0
28.		0.0	0.0	0.0	0.0	0.0	0.0	0.0
29.		45.4	35.4	54.6	69.0	28.1	51.0	51.0
30.		100.0	100.0	100.0	100.0	100.0	100.0	100.0
31.		0.0	0.0	0.0	0.0	0.0	0.0	0.0
32.		0.0	0.0	0.0	0.0	0.0	5.1	0.0
33.		28.9	20.2	29.9	29.9	27.1	21.4	20.0
34.		0.0	0.0	0.0	0.0	0.0	0.0	0.0
35.		7.2	7.1	14.4	14.9	12.5	14.3	21.0
36.		0.0	0.0	0.0	2.3	0.0	1.0	1.0
37.		7.2	3.0	2.1	6.9	3.1	4.1	0.0
38.		0.0	0.0	0.0	0.0	0.0	0.0	0.0
39.		0.0	0.0	0.0	0.0	0.0	0.0	0.0
40.		0.0	0.0	0.0	0.0	0.0	0.0	0.0
41.		0.0	0.0	0.0	0.0	0.0	0.0	0.0
42.		5.2	11.1	15.5	26.4	12.5	2.0	2.0
43.		64.9	78.8	62.9	69.0	77.1	74.5	77.0
44.		72.2	68.7	70.1	63.2	54.2	68.4	60.0

Table C9. Continued

Character Number	N=	UNTD 97	VEH-7 99	BUDR-L7 97	BUDR-H7 87	VEH-8 96	BUDR-L8 98	BUDR-H8 100
45.		25.8	23.2	26.8	24.1	13.5	13.3	23.0
46.		1.0	0.0	0.0	8.0	0.0	0.0	1.0
47.		35.1	22.2	26.8	11.5	10.4	16.3	20.0
48.		0.0	1.0	2.1	12.6	1.0	2.0	4.0
49.		17.5	10.1	5.2	8.0	5.2	9.2	12.0
50.		9.3	1.0	6.2	2.3	0.0	0.0	3.0
51.		2.1	2.0	2.1	10.3	4.2	1.0	3.0
52.		74.2	56.6	57.7	43.7	63.5	54.1	54.0
53.		6.2	2.0	1.0	12.6	7.3	6.1	2.0
54.		18.6	31.3	26.8	27.6	20.8	27.6	18.0
55.		0.0	1.0	0.0	4.6	1.0	2.0	1.0
56.		0.0	4.0	0.0	0.0	0.0	0.0	2.0
57.		0.0	0.0	1.0	1.1	0.0	0.0	0.0
58.		0.0	0.0	0.0	0.0	0.0	0.0	0.0
59.		1.0	0.0	1.0	10.3	0.0	2.0	8.0
60.		44.3	34.3	45.4	44.8	37.5	33.7	29.0
61.		0.0	0.0	0.0	3.4	0.0	3.1	4.0
62.		8.2	6.1	6.2	8.0	5.2	6.1	9.0
63.		1.0	4.0	1.0	1.1	1.0	0.0	3.0
64.		16.5	6.1	19.6	9.2	13.5	11.2	11.0
65.		0.0	1.0	3.1	3.4	3.1	1.0	1.0
66.		1.0	0.0	0.0	0.0	0.0	0.0	0.0
67.		97.9	88.9	92.8	82.8	88.5	92.9	87.0
68.		3.1	2.0	1.0	12.6	1.0	4.1	14.0
69.		1.0	5.1	1.0	6.9	1.0	1.0	6.0
70.		53.6	43.4	51.5	90.8	53.1	48.0	93.0
71.		0.0	0.0	0.0	3.4	0.0	0.0	13.0
72.		16.5	18.2	25.8	21.8	10.4	17.3	17.0
73.		43.3	40.4	27.8	35.6	41.7	26.5	13.0
74.		27.8	16.2	18.6	4.6	15.6	17.3	4.0
75.		3.1	4.0	8.2	10.3	7.3	8.2	12.0
76.		0.0	0.0	0.0	0.0	0.0	0.0	0.0
77.		0.0	0.0	1.0	3.4	0.0	0.0	4.0
78.		0.0	0.0	1.0	10.3	0.0	2.0	5.0
79.		5.2	4.0	8.2	4.6	2.1	0.0	3.0
80.		1.0	0.0	0.0	1.1	1.0	0.0	0.0
81.		3.1	1.0	1.0	3.4	3.1	0.0	1.0
82.		7.2	3.0	15.5	9.2	7.3	11.2	7.0
83.		0.0	0.0	0.0	0.0	0.0	0.0	0.0
84.		0.0	0.0	0.0	0.0	0.0	0.0	0.0
85.		0.0	0.0	0.0	0.0	0.0	0.0	0.0
86.		0.0	0.0	0.0	0.0	0.0	0.0	0.0
87.		4.1	3.0	1.0	1.1	3.1	1.0	3.0
88.		4.1	5.1	2.1	3.4	6.2	4.1	6.0

Table C10. Frequencies of occurrence of each skeletal variant in treatments with Bromodeoxyuridine on day 9 of gestation, an experiment added to the second BUDR series

Character Number		UNTD	VEH-9	BUDR-L9	BUDR-H9
	N=	97	93	96	92
1.		0.0	0.0	0.0	0.0
2.		0.0	0.0	0.0	0.0
3.		0.0	0.0	0.0	0.0
4.		0.0	0.0	0.0	0.0
5.		0.0	0.0	0.0	0.0
6.		33.0	30.1	34.4	34.8
7.		0.0	0.0	0.0	0.0
8.		66.0	73.1	68.7	67.4
9.		0.0	1.1	5.2	2.2
10.		14.4	17.2	13.5	12.0
11.		1.0	0.0	1.0	1.1
12.		0.0	0.0	0.0	0.0
13.		0.0	0.0	0.0	0.0
14.		9.3	8.6	13.5	28.3
15.		1.0	1.1	2.1	0.0
16.		30.9	19.4	26.0	43.5
17.		0.0	0.0	0.0	0.0
18.		1.0	0.0	0.0	0.0
19.		0.0	0.0	0.0	0.0
20.		4.1	3.2	3.1	5.4
21.		22.7	30.1	26.0	34.8
22.		55.7	61.3	62.5	55.4
23.		2.1	4.3	6.2	5.4
24.		0.0	0.0	0.0	0.0
25.		0.0	0.0	0.0	0.0
26.		24.7	23.7	26.0	68.5
27.		33.0	21.5	27.1	20.7
28.		0.0	0.0	0.0	0.0
29.		45.4	58.1	43.7	48.9
30.		100.0	100.0	100.0	100.0
31.		0.0	0.0	0.0	0.0
32.		0.0	0.0	0.0	0.0
33.		28.9	20.4	28.1	29.3
34.		0.0	0.0	0.0	0.0
35.		7.2	15.1	11.5	9.8
36.		0.0	0.0	0.0	2.2
37.		7.2	5.4	8.3	2.2
38.		0.0	0.0	0.0	0.0
39.		0.0	0.0	0.0	0.0
40.		0.0	0.0	0.0	0.0
41.		0.0	0.0	0.0	0.0
42.		5.2	5.4	9.4	6.5
43.		64.9	63.4	78.1	79.3
44.		72.2	62.4	74.0	78.3

Table C10. Continued

Character Number N=	UNTD 97	VEH-9 93	BUDR-L9 96	BUDR-H9 92
45.	25.8	40.9	34.4	25.0
46.	1.0	0.0	0.0	0.0
47.	35.1	15.1	25.0	15.2
48.	0.0	2.2	3.1	0.0
49.	17.5	5.4	8.3	15.2
50.	9.3	3.2	1.0	6.5
51.	2.1	1.1	0.0	1.1
52.	74.2	67.7	57.3	64.1
53.	6.2	5.4	7.3	3.3
54.	18.6	30.1	30.2	31.5
55.	0.0	3.2	1.0	0.0
56.	0.0	3.2	1.0	0.0
57.	0.0	0.0	1.0	1.1
58.	0.0	0.0	0.0	0.0
59.	1.0	0.0	1.0	0.0
60.	44.3	49.5	47.9	44.6
61.	0.0	0.0	0.0	0.0
62.	8.2	7.5	3.1	2.2
63.	1.0	0.0	0.0	1.1
64.	16.5	8.6	2.1	4.3
65.	0.0	0.0	0.0	0.0
66.	1.0	0.0	0.0	0.0
67.	97.9	79.6	76.0	87.0
68.	3.1	2.2	1.0	2.2
69.	1.0	1.1	2.1	3.3
70.	53.6	45.2	51.0	62.0
71.	0.0	0.0	0.0	1.1
72.	16.5	35.5	37.5	21.7
73.	43.3	26.9	27.1	20.7
74.	27.8	19.4	14.6	7.6
75.	3.1	6.5	17.7	31.5
76.	0.0	0.0	0.0	0.0
77.	0.0	2.2	0.0	27.2
78.	0.0	0.0	0.0	16.3
79.	5.2	9.7	4.2	13.0
80.	1.0	0.0	0.0	13.0
81.	3.1	1.1	0.0	16.3
82.	7.2	9.7	3.1	5.4
83.	0.0	0.0	0.0	1.1
84.	0.0	0.0	0.0	0.0
85.	0.0	0.0	0.0	0.0
86.	0.0	0.0	0.0	0.0
87.	4.1	0.0	0.0	0.0
88.	4.1	3.2	3.1	7.6

Table C11. Frequencies of occurrence of each skeletal variant in Diphenylhydantoin experiments.

			Diphenylhydantoin (mg/kg/day)			
Character	UNTD	VEH	6.25mg	12.5mg	25mg	50mg
Number N=	98	100	98	98	99	95
1.	0.0	0.0	0.0	0.0	0.0	0.0
2.	0.0	0.0	0.0	0.0	0.0	0.0
3.	0.0	1.0	0.0	0.0	0.0	0.0
4.	0.0	0.0	0.0	0.0	0.0	0.0
5.	0.0	0.0	0.0	0.0	0.0	0.0
6.	19.4	24.0	34.7	22.4	32.3	43.2
7.	0.0	0.0	0.0	0.0	0.0	0.0
8.	68.4	68.0	75.5	72.4	73.7	72.6
9.	2.0	6.0	3.1	2.0	1.0	2.1
10.	18.4	9.0	15.3	16.3	25.3	14.7
11.	0.0	2.0	0.0	1.0	1.0	0.0
12.	0.0	0.0	0.0	0.0	0.0	0.0
13.	0.0	0.0	0.0	0.0	0.0	0.0
14.	5.1	16.0	3.1	10.2	15.2	21.1
15.	0.0	1.0	0.0	0.0	0.0	0.0
16.	55.1	61.0	66.3	50.0	51.5	45.3
17.	0.0	0.0	0.0	0.0	0.0	1.1
18.	1.0	2.0	1.0	0.0	0.0	0.0
19.	0.0	0.0	0.0	0.0	0.0	0.0
20.	1.0	4.0	2.0	2.0	0.0	0.0
21.	9.2	22.0	14.3	23.5	9.1	18.9
22.	53.1	50.0	62.2	51.0	55.6	51.6
23.	4.1	1.0	2.0	2.0	1.0	4.2
24.	0.0	0.0	0.0	0.0	0.0	0.0
25.	0.0	0.0	0.0	0.0	0.0	0.0
26.	29.6	33.0	22.4	22.4	13.1	13.7
27.	20.4	44.0	26.5	27.6	20.2	38.9
28.	0.0	0.0	0.0	0.0	0.0	0.0
29.	53.1	47.0	54.1	60.2	44.4	51.6
30.	100.0	100.0	100.0	100.0	100.0	100.0
31.	0.0	0.0	0.0	0.0	0.0	0.0
32.	0.0	0.0	1.0	0.0	0.0	1.1
33.	15.3	19.0	21.4	16.3	24.2	32.6
34.	0.0	0.0	0.0	0.0	0.0	0.0
35.	17.3	20.0	23.5	16.3	18.2	16.8
36.	3.1	1.0	1.0	1.0	3.0	1.1
37.	3.1	4.0	6.1	4.1	6.1	2.1
38.	0.0	0.0	0.0	0.0	0.0	0.0
39.	0.0	0.0	0.0	0.0	0.0	0.0
40.	0.0	0.0	0.0	0.0	0.0	0.0
41.	0.0	0.0	0.0	0.0	0.0	0.0
42.	14.3	10.0	8.2	9.2	9.1	5.3
43.	62.2	72.0	69.4	74.5	80.8	76.8
44.	60.2	69.0	64.3	63.3	61.6	61.1

Table C11. Continued

Character Number			Diphenylhydantoin (mg/kg/day)			
	UNTD	VEH	6.25mg	12.5mg	25mg	50mg
N=	98	100	98	98	99	95
45.	30.6	26.0	21.4	27.6	33.3	32.6
46.	0.0	0.0	0.0	0.0	0.0	0.0
47.	16.3	12.0	7.1	20.4	14.1	36.8
48.	2.0	3.0	1.0	9.2	2.0	4.2
49.	2.0	3.0	4.1	6.1	7.1	7.4
50.	1.0	4.0	4.1	6.1	2.0	2.1
51.	4.1	3.0	5.1	2.0	2.0	2.1
52.	79.6	61.0	62.2	57.1	73.7	71.6
53.	6.1	3.0	5.1	2.0	4.0	2.1
54.	21.4	41.0	31.6	40.8	32.3	40.0
55.	1.0	0.0	2.0	0.0	0.0	0.0
56.	1.0	0.0	1.0	3.1	2.0	1.1
57.	1.0	0.0	1.0	0.0	0.0	0.0
58.	0.0	0.0	0.0	1.0	0.0	1.1
59.	3.1	0.0	0.0	0.0	2.0	3.2
60.	44.9	38.0	52.0	53.1	45.5	60.0
61.	1.0	1.0	1.0	3.1	0.0	2.1
62.	2.0	1.0	4.1	0.0	0.0	2.1
63.	1.0	2.0	0.0	1.0	2.0	1.1
64.	8.2	11.0	16.3	12.2	16.2	2.1
65.	0.0	2.0	0.0	0.0	0.0	0.0
66.	0.0	0.0	0.0	0.0	0.0	0.0
67.	85.7	88.0	87.8	80.6	84.8	77.9
68.	1.0	1.0	2.0	6.1	1.0	7.4
69.	2.0	2.0	1.0	3.1	3.0	0.0
70.	32.7	64.0	35.7	59.2	41.4	36.8
71.	0.0	0.0	0.0	0.0	0.0	0.0
72.	28.6	21.0	31.6	27.6	35.4	47.4
73.	37.8	32.0	28.6	40.8	41.4	26.3
74.	23.5	15.0	33.7	22.4	21.2	13.7
75.	11.2	11.0	5.1	12.2	3.0	18.9
76.	1.0	0.0	0.0	1.0	0.0	0.0
77.	0.0	0.0	0.0	1.0	0.0	0.0
78.	0.0	1.0	1.0	1.0	0.0	2.1
79.	1.0	2.0	1.0	1.0	5.1	5.3
80.	0.0	0.0	0.0	1.0	0.0	1.1
81.	0.0	0.0	1.0	1.0	1.0	2.1
82.	8.2	5.0	8.2	9.2	1.0	9.5
83.	0.0	0.0	0.0	0.0	0.0	0.0
84.	0.0	0.0	0.0	0.0	0.0	0.0
85.	0.0	0.0	0.0	0.0	0.0	0.0
86.	0.0	0.0	0.0	0.0	0.0	0.0
87.	1.0	0.0	1.0	1.0	1.0	2.1
88.	10.2	5.0	2.0	4.1	2.0	6.3

Table C12. Frequencies of occurrence of each skeletal variant in Cortisone experiments.

			Cortisone (mg/mouse/day)			
Character Number N=	UNTD 94	VEH 98	0.15mg 98	0.30mg 97	0.60mg 98	1.20mg 99
1.	0.0	0.0	0.0	0.0	0.0	0.0
2.	0.0	0.0	0.0	0.0	0.0	0.0
3.	0.0	0.0	2.0	0.0	0.0	1.0
4.	0.0	0.0	0.0	0.0	0.0	0.0
5.	0.0	0.0	0.0	0.0	0.0	0.0
6.	20.2	22.4	21.4	24.7	22.4	22.2
7.	0.0	0.0	0.0	0.0	0.0	0.0
8.	80.9	74.5	58.2	82.5	79.6	78.8
9.	4.3	0.0	8.2	2.1	4.1	0.0
10.	29.8	17.3	10.2	17.5	12.2	9.1
11.	2.1	1.0	0.0	2.1	0.0	7.1
12.	0.0	0.0	0.0	0.0	0.0	0.0
13.	0.0	0.0	0.0	0.0	0.0	0.0
14.	9.6	6.1	5.1	1.0	3.1	6.1
15.	1.1	1.0	0.0	0.0	0.0	1.0
16.	57.4	66.3	70.4	84.5	94.9	96.0
17.	0.0	0.0	0.0	0.0	0.0	0.0
18.	1.1	1.0	1.0	3.1	4.1	1.0
19.	0.0	0.0	0.0	0.0	0.0	0.0
20.	0.0	0.0	0.0	0.0	0.0	0.0
21.	8.5	12.2	9.2	11.3	13.3	6.1
22.	56.4	52.0	64.3	55.7	48.0	46.5
23.	5.3	6.1	4.1	4.1	5.1	8.1
24.	0.0	0.0	0.0	0.0	0.0	0.0
25.	0.0	0.0	1.0	0.0	0.0	0.0
26.	55.3	56.1	45.9	59.8	81.6	78.8
27.	59.6	54.1	41.8	68.0	56.1	60.6
28.	0.0	0.0	0.0	0.0	0.0	0.0
29.	47.9	57.1	45.9	51.5	43.9	45.5
30.	100.0	99.0	100.0	100.0	100.0	100.0
31.	0.0	0.0	0.0	0.0	0.0	0.0
32.	0.0	0.0	1.0	0.0	0.0	0.0
33.	46.8	27.6	43.9	62.9	54.1	41.4
34.	0.0	0.0	0.0	0.0	0.0	0.0
35.	14.9	28.6	23.5	19.6	24.5	30.3
36.	3.2	2.0	3.1	4.1	9.2	6.1
37.	2.1	1.0	1.0	0.0	0.0	2.0
38.	0.0	0.0	0.0	0.0	0.0	0.0
39.	0.0	0.0	0.0	0.0	0.0	0.0
40.	0.0	1.0	0.0	0.0	0.0	0.0
41.	0.0	0.0	0.0	0.0	0.0	0.0
42.	2.1	2.0	4.1	2.1	0.0	3.0
43.	74.5	72.4	56.1	73.2	79.6	61.6
44.	37.2	60.2	51.0	49.5	55.1	51.5

Table C12. Continued

				Cortisone (mg/mouse/day)			
Character Number	N=	UNTD 94	VEH 98	0.15mg 98	0.30mg 97	0.60mg 98	1.20mg 99
45.		19.1	19.4	19.4	29.9	22.4	27.3
46.		0.0	1.0	0.0	0.0	0.0	3.0
47.		7.4	4.1	4.1	7.2	11.2	4.0
48.		12.8	3.1	6.1	4.1	6.1	6.1
49.		2.1	2.0	1.0	0.0	1.0	2.0
50.		6.4	1.0	0.0	1.0	2.0	7.1
51.		14.9	14.3	15.3	15.5	14.3	4.0
52.		40.4	66.3	50.0	43.3	48.0	46.5
53.		19.1	10.2	7.1	8.2	9.2	8.1
54.		59.6	50.0	41.8	47.4	39.8	41.4
55.		0.0	2.0	0.0	1.0	3.1	0.0
56.		2.1	2.0	0.0	0.0	2.0	1.0
57.		0.0	0.0	0.0	0.0	0.0	0.0
58.		0.0	1.0	0.0	0.0	0.0	0.0
59.		1.1	0.0	0.0	0.0	1.0	0.0
60.		47.9	52.0	51.0	49.5	48.0	43.4
61.		0.0	1.0	0.0	1.0	2.0	1.0
62.		6.4	6.1	5.1	3.1	5.1	4.0
63.		2.1	1.0	3.1	0.0	2.0	0.0
64.		0.0	0.0	2.0	0.0	0.0	2.0
65.		5.3	1.0	2.0	1.0	2.0	0.0
66.		0.0	0.0	1.0	0.0	0.0	0.0
67.		78.7	76.5	79.6	68.0	75.5	77.8
68.		2.1	12.2	2.0	13.4	23.5	23.2
69.		2.1	1.0	1.0	3.1	4.1	6.1
70.		34.0	23.5	26.5	13.4	23.5	13.1
71.		0.0	0.0	0.0	1.0	1.0	0.0
72.		43.6	36.7	33.7	25.8	34.7	43.4
73.		37.2	54.1	46.9	60.8	63.3	55.6
74.		27.7	34.7	29.6	42.3	45.9	22.2
75.		8.5	3.1	5.1	4.1	7.1	12.1
76.		1.1	7.1	3.1	3.1	3.1	4.0
77.		1.1	0.0	0.0	0.0	0.0	0.0
78.		0.0	0.0	0.0	0.0	0.0	0.0
79.		0.0	0.0	0.0	0.0	0.0	1.0
80.		0.0	0.0	0.0	0.0	0.0	0.0
81.		0.0	0.0	0.0	0.0	0.0	0.0
82.		6.4	16.3	12.2	21.6	20.4	18.2
83.		2.1	1.0	1.0	2.1	3.1	1.0
84.		0.0	0.0	0.0	0.0	0.0	0.0
85.		0.0	0.0	0.0	0.0	0.0	0.0
86.		19.1	9.2	7.1	7.2	10.2	16.2
87.		1.1	0.0	3.1	1.0	2.0	6.1
88.		2.1	3.1	4.1	2.1	2.0	2.0

Table C13. Frequencies of occurrence of each skeletal variant among untreated CD-1 mice from separately purchased groups of animals used in the experimental series indicated.

		TFLT	TCAP	MBDM	TB-I	ACZM-I	BUDR-I	AZ/TB-II	Average of all
	N =	94	92	104	100	98	98	100	7 Expts.
1.		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2.		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3.		0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.1
4.		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5.		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6.		44.7	28.3	55.8	63.0	31.6	28.6	37.0	41.3
7.		1.1	1.1	0.0	0.0	0.0	0.0	0.0	0.3
8.		94.7	69.6	55.8	65.0	76.5	80.6	69.0	73.0
9.		1.1	6.5	14.4	11.0	1.0	1.0	9.0	6.3
10.		40.4	15.2	27.9	18.0	23.5	15.3	28.0	24.0
11.		4.3	12.0	1.0	2.0	1.0	3.1	1.0	3.5
12.		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
13.		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
14.		3.2	3.3	9.6	18.0	9.2	9.2	11.0	9.1
15.		0.0	0.0	1.0	1.0	0.0	0.0	0.0	0.3
16.		23.4	12.0	37.5	20.0	44.9	73.5	48.0	37.0
17.		0.0	1.1	0.0	0.0	0.0	0.0	0.0	0.2
18.		0.0	3.3	7.7	0.0	1.0	0.0	0.0	1.7
19.		6.4	2.2	0.0	0.0	0.0	0.0	0.0	1.2
20.		1.1	4.3	4.8	3.0	6.1	2.0	6.0	3.9
21.		24.5	13.0	2.9	31.0	27.6	17.3	25.0	20.2
22.		75.5	88.0	79.8	61.0	71.4	61.2	49.0	69.4
23.		8.5	9.8	7.7	8.0	3.1	6.1	13.0	8.0
24.		1.1	0.0	0.0	0.0	0.0	0.0	0.0	0.2
25.		8.5	13.0	6.7	0.0	0.0	1.0	1.0	4.3
26.		13.8	40.2	14.4	29.0	25.5	23.5	23.0	24.2
27.		51.1	34.8	57.7	26.0	32.7	69.4	40.0	44.5
28.		0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.1
29.		57.4	23.9	53.8	40.0	58.2	45.9	41.0	45.8
30.		76.6	64.1	100.0	100.0	100.0	91.8	100.0	90.4
31.		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
32.		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
33.		36.2	15.2	42.3	33.0	25.5	39.8	31.0	31.9
34.		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
35.		36.2	5.4	12.5	8.0	3.1	8.2	21.0	13.5
36.		1.1	3.3	3.8	5.0	3.1	0.0	6.0	3.2
37.		2.1	0.0	1.9	51.0	37.8	58.2	23.0	24.9
38.		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
39.		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
40.		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
41.		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
42.		5.3	4.3	3.8	15.0	7.1	6.1	9.0	7.3
43.		89.4	82.6	91.3	74.0	75.5	75.5	83.0	81.6
44.		0.0	4.3	94.2	90.0	81.6	46.9	49.0	52.3

Table C13. Continued

N =	TFLT 94	TCAP 92	MBDM 104	TB-I 100	ACZM-I 98	BUDR-I 98	AZ/TB-II 100	Average of all 7 Expts.
45.	4.3	23.9	32.7	5.0	20.4	10.2	16.0	16.1
46.	1.1	1.1	0.0	0.0	0.0	0.0	0.0	0.3
47.	8.5	10.9	4.8	12.0	11.2	4.1	9.0	8.6
48.	3.2	7.6	1.9	4.0	1.0	10.2	11.0	5.6
49.	3.2	14.1	1.0	1.0	1.0	0.0	3.0	3.3
50.	0.0	0.0	13.5	0.0	6.1	1.0	2.0	3.2
51.	31.9	4.3	1.0	2.0	1.0	0.0	0.0	5.7
52.	6.4	34.8	93.3	64.0	90.8	78.6	82.0	64.3
53.	7.4	3.3	5.8	10.0	10.2	22.4	8.0	9.6
54.	57.4	25.0	53.8	36.0	45.9	42.9	26.0	41.0
55.	1.1	1.1	0.0	1.0	0.0	1.0	1.0	0.7
56.	1.1	1.1	1.9	3.0	1.0	0.0	2.0	1.4
57.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
58.	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.1
59.	0.0	0.0	1.9	0.0	2.0	0.0	0.0	0.6
60.	54.3	40.2	93.3	81.0	79.6	61.2	51.0	65.8
61.	1.1	0.0	1.0	0.0	1.0	1.0	1.0	0.7
62.	3.2	1.1	1.0	2.0	2.0	12.2	2.0	3.4
63.	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.1
64.	10.6	8.7	0.0	2.0	7.1	4.1	11.0	6.2
65.	1.1	0.0	0.0	0.0	0.0	0.0	0.0	0.2
66.	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.1
67.	75.5	85.9	99.0	94.0	83.7	74.5	99.0	87.4
68.	10.6	16.3	4.8	5.0	2.0	6.1	1.0	6.6
69.	0.0	0.0	1.0	1.0	2.0	4.1	0.0	1.2
70.	22.3	28.3	37.5	43.0	24.5	36.7	68.0	37.2
71.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
72.	50.0	18.5	17.3	20.0	16.3	27.6	14.0	23.4
73.	71.3	50.0	91.3	49.0	66.3	42.9	27.0	56.8
74.	55.3	56.5	20.2	13.0	32.7	45.9	15.0	34.1
75.	10.6	10.9	32.7	27.0	5.1	6.1	21.0	16.2
76.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
77.	0.0	0.0	0.0	0.0	0.0	0.0	2.0	0.3
78.	0.0	0.0	0.0	2.0	0.0	0.0	0.0	0.3
79.	23.4	4.3	9.6	12.0	3.1	2.0	0.0	7.8
80.	1.1	0.0	1.0	0.0	0.0	0.0	1.0	0.4
81.	1.1	2.2	1.0	8.0	1.0	0.0	1.0	2.0
82.	3.2	9.8	51.0	72.0	11.2	19.4	11.0	25.4
83.	31.9	1.1	1.0	1.0	2.0	0.0	0.0	5.3
84.	1.1	0.0	0.0	0.0	0.0	0.0	1.0	0.3
85.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
86.	0.0	1.1	0.0	1.0	0.0	0.0	1.0	0.4
87.	19.1	12.0	0.0	4.0	2.0	1.0	2.0	5.7
88.	1.1	2.2	4.8	5.0	5.1	3.1	4.0	3.6

Table C14. Frequencies of occurrence of each variant in Vehicle controls in several series of experiments.

	TFLT	TCAP	MBDM		TB-I	ACZM-I		BUDR-I		ACZM-II		TB-II
	Corn Oil	CMC	Corn Oil	CMC	NaCl	H_2O-8	H_2O-9x3	H_2O-7	H_2O-8	H_2O-8	H_2O-9x3	NaCl
N=	95	91	101	100	100	97	100	97	98	100	83	97
1.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.0	0.0	0.0
4.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6.	50.5	33.7	51.5	58.0	58.0	25.8	31.0	28.9	16.3	33.0	31.3	38.1
7.	1.1	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0
8.	94.7	71.6	59.4	59.0	59.0	78.4	66.0	84.5	84.7	60.0	59.0	66.0
9.	3.2	3.2	17.8	11.0	17.0	2.1	0.0	1.0	3.1	4.0	3.6	8.2
10.	17.9	12.6	23.8	22.0	19.0	29.9	21.0	11.3	15.3	23.0	25.3	9.3
11.	18.9	12.6	0.0	0.0	0.0	2.1	5.0	3.1	2.0	11.0	2.4	2.1
12.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
13.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
14.	8.4	17.9	22.8	27.0	11.0	9.3	5.0	15.5	12.2	17.0	18.1	11.3
15.	2.1	0.0	0.0	3.0	0.0	1.0	1.0	1.0	0.0	0.0	1.2	1.0
16.	24.2	37.9	60.4	47.0	19.0	36.1	34.0	52.6	65.3	47.0	62.7	67.0
17.	0.0	1.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
18.	0.0	4.2	2.0	6.0	1.0	1.0	0.0	0.0	1.0	2.0	0.0	0.0
19.	3.2	6.3	0.0	0.0	1.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0
20.	3.2	1.1	7.9	2.0	3.0	2.1	5.0	2.1	2.0	3.0	8.4	2.1
21.	24.2	24.2	26.7	20.0	19.0	17.5	17.0	15.5	18.4	15.0	15.7	24.7
22.	69.5	81.1	78.2	65.0	75.0	58.8	47.0	55.7	67.3	58.0	48.2	53.6
23.	10.5	11.6	7.9	9.0	6.0	8.2	12.0	10.3	4.1	9.0	16.9	11.3
24.	1.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
25.	9.5	10.5	5.0	7.0	0.0	0.0	0.0	1.0	1.0	0.0	1.2	1.0
26.	30.5	45.3	23.8	23.0	36.0	19.6	20.0	33.0	39.8	21.0	25.3	16.5
27.	46.3	33.7	50.5	57.0	28.0	40.2	37.0	67.0	62.2	41.0	27.7	38.1
28.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
29.	48.4	35.8	52.5	53.0	40.0	57.7	34.0	44.3	49.0	38.0	34.9	41.2
30.	77.9	69.5	100.0	100.0	100.0	100.0	99.0	70.1	76.5	100.0	100.0	97.9
31.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
32.	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
33.	37.9	34.7	38.6	40.0	21.0	22.7	41.0	38.1	39.8	40.0	37.3	23.7
34.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
35.	22.1	8.4	5.0	4.0	17.0	10.3	11.0	12.4	9.2	11.0	9.6	17.5
36.	1.1	0.0	3.0	10.0	0.0	2.1	1.0	2.1	3.1	7.0	2.4	5.2
37.	3.2	1.1	3.0	1.0	47.0	48.5	43.0	40.2	35.7	21.0	21.7	24.7
38.	1.1	3.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
39.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
40.	0.0	1.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
41.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
42.	12.6	4.2	5.9	4.0	8.0	12.4	23.0	9.3	4.1	28.0	7.2	13.4

Table C14. Continued

43.	92.6	67.4	89.1	93.0	72.0	79.4	79.0	67.0	69.4	81.0	73.5	71.1
44.	0.0	3.2	89.1	88.0	91.0	74.2	72.0	47.4	45.9	48.0	48.2	59.8
45.	10.5	8.4	39.6	34.0	8.0	27.8	36.0	18.6	19.4	19.0	26.5	5.2
46.	1.1	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	1.2	0.0
47.	7.4	16.8	5.0	6.0	8.0	7.2	8.0	15.5	19.4	9.0	2.4	13.4
48.	3.2	16.8	8.9	2.0	5.0	3.1	2.0	5.2	3.1	9.0	2.4	1.0
49.	1.1	22.1	1.0	0.0	1.0	12.4	5.0	7.2	6.1	3.0	1.2	8.2
50.	0.0	0.0	5.0	9.0	4.0	5.2	0.0	0.0	5.1	2.0	4.8	1.0
51.	40.0	6.3	0.0	0.0	2.0	4.1	3.0	0.0	0.0	3.0	3.6	0.0
52.	3.2	32.6	84.2	78.0	71.0	76.3	78.0	82.5	85.7	84.0	77.1	75.3
53.	22.1	2.1	8.9	9.0	6.0	7.2	13.0	12.4	11.2	7.0	4.8	4.1
54.	49.5	29.5	61.4	44.0	27.0	57.7	22.0	37.1	38.8	35.0	39.8	37.1
55.	0.0	1.1	0.0	1.0	0.0	0.0	0.0	0.0	2.0	0.0	0.0	0.0
56.	1.1	0.0	0.0	1.0	3.0	0.0	0.0	0.0	1.0	1.0	0.0	0.0
57.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0
58.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
59.	0.0	0.0	0.0	1.0	0.0	1.0	0.0	0.0	0.0	2.0	0.0	1.0
60.	53.7	43.2	95.0	93.0	93.0	90.7	79.0	73.2	73.5	41.0	61.4	35.1
61.	2.1	2.1	0.0	1.0	0.0	0.0	0.0	0.0	0.0	2.0	1.2	0.0
62.	0.0	3.2	2.0	0.0	2.0	3.1	3.0	2.1	5.1	3.0	0.0	2.1
63.	6.3	3.2	1.0	0.0	1.0	0.0	0.0	1.0	0.0	0.0	0.0	2.1
64.	2.1	4.2	4.0	0.0	5.0	8.2	3.0	3.1	3.1	15.0	13.3	7.2
65.	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
66.	2.1	1.1	1.0	0.0	0.0	0.0	0.0	1.0	0.0	3.0	0.0	0.0
67.	64.2	81.1	98.0	96.0	88.0	82.5	89.0	88.7	84.7	80.0	83.1	75.3
68.	13.7	6.3	3.0	6.0	1.0	1.0	3.0	3.1	5.1	3.0	4.8	1.0
69.	3.2	3.2	0.0	1.0	4.0	0.0	5.0	7.2	6.1	1.0	1.2	2.1
70.	24.2	55.8	40.6	26.0	39.0	25.8	31.0	20.6	28.6	67.0	45.8	52.6
71.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
72.	41.1	18.9	19.8	30.0	25.0	13.4	17.0	35.1	24.5	19.0	22.9	19.6
73.	69.5	41.1	99.0	95.0	66.0	52.6	58.0	52.6	51.0	26.0	27.7	38.1
74.	45.3	43.2	12.9	27.0	26.0	38.1	19.0	35.1	41.8	18.0	7.2	24.7
75.	15.8	5.3	18.8	12.0	21.0	20.6	14.0	4.1	5.1	17.0	13.3	1.0
76.	0.0	1.1	0.0	0.0	0.0	0.0	0.0	3.1	1.0	0.0	0.0	1.0
77.	1.1	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	1.2	1.0
78.	0.0	4.2	1.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0
79.	21.1	6.3	15.8	19.0	12.0	2.1	6.0	0.0	4.1	1.0	9.6	3.1
80.	0.0	0.0	0.0	1.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
81.	1.1	0.0	0.0	1.0	8.0	2.1	6.0	0.0	1.0	2.0	3.6	1.0
82.	18.9	9.5	56.4	62.0	77.0	11.3	3.0	20.6	12.2	4.0	4.8	5.2
83.	28.4	3.2	2.0	1.0	1.0	0.0	0.0	1.0	0.0	0.0	1.2	0.0
84.	1.1	0.0	0.0	2.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0
85.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
86.	0.0	0.0	2.0	0.0	1.0	0.0	0.0	0.0	2.0	0.0	0.0	0.0
87.	25.3	12.6	4.0	5.0	9.0	5.2	7.0	4.1	5.1	1.0	1.2	0.0
88.	1.1	1.1	0.0	2.0	9.0	3.1	5.0	2.1	5.1	5.0	3.6	2.1

Table C14. Continued

Legend:

There is a slight unresolved discrepancy in the computer print outs of the vehicle frequencies in the TCAP series between this Table and Table C2.

Note that the frequencies in the two vehicle groups which occur in the same series (MBDM, ACZM-I, BUDR-I, ACZM-II) are very similar in each case, despite the fact that different vehicles (MBDM), different days of treatment (BUDR) or different treatment regimes (ACZM) are employed. These data are not useful for comparing the effects of the same vehicle in different "batches" of mice since untreated frequencies also differ.

Appendix D. Frequencies of Occurrence (%) of Each Skeletal Variant in Several Generations of a Four-Way Outcross Mouse Population Selected for Susceptibility or Resistance to Trypan Blue Teratogenesis.

Articulated, alizarin-stained skeletons of varying ages were available, from a series of experiments involving maximally outbred mice which were descendents of a cross between C57BL, CBA, C3H, and A-strain mice (CBHA), which had been selected for differing responses to trypan blue teratogenesis. (See S.L. Beck 1971, J. Exp. Zool. 177: 479-492 and the text of the present report for additional details). These animals were examined for the 88 characters under investigation as a parallel study with a different group of mice.

Tables 1-10 present the numbers of specimens (N) in each group and the frequencies of each variant.

A,B = the two halves of the reciprocal crosses

A2C, B2C = Untreated animals

A2T, B2T = Animals from treated litters

S = CBHA-S = Selected, susceptible line

R = CBHA-R = Selected, resistant line

C = CBHA-CT = A treated subsample from an unselected line, not treated in any earlier generation

U = CBHA-C = Representatives of the unselected control line, the present sample also being untreated

6SC, 6RC = Untreated individuals in generation 6, in which selection had been relaxed

G = Gen = Generation number (followed by 2,3,6,7,9 as appropriate)

Since G6 was an untreated generation (except for a small group of CBHA-S) only these generations are averaged in Table 9.

Tables 11-13 present differences between each treated group and untreated controls in each generation for which data are available. G6RC (untreated) is presented in Table 12, but not averaged. Averages are unweighted.

$\bar{X}$ = mean of differences taking direction of difference into account

$\bar{X}$ (Abso) = mean of the absolute value of the differences

Character numbers correspond with those in Table 3 of the body of the report.

Table D1. Frequencies of occurrence of each skeletal variant in the first generation of a four-way outcross mouse population (C57BL, CBA, C3H, A) treated with trypan blue.

Character Number N=	A2C 36	B2C 34	A2T 78	B2T 100	Character Number	A2C 36	B2C 34	A2T 78	B2T 100
1.	0.0	0.0	6.4	7.0	45.	2.8	0.0	5.1	6.0
2.	0.0	2.9	17.9	11.0	46.	0.0	0.0	6.4	5.0
3.	0.0	0.0	0.0	0.0	47.	0.0	0.0	3.8	0.0
4.	0.0	0.0	0.0	0.0	48.	2.8	0.0	1.3	4.0
5.	0.0	0.0	0.0	0.0	49.	0.0	0.0	5.1	3.0
6.	8.3	8.8	5.1	7.0	50.	0.0	8.8	7.7	0.0
7.	0.0	0.0	0.0	0.0	51.	22.2	23.5	52.6	55.0
8.	19.4	38.2	34.6	32.0	52.	2.8	5.9	12.8	10.0
9.	16.7	29.4	10.3	15.0	53.	11.1	11.8	11.5	4.0
10.	13.9	20.6	34.6	26.0	54.	11.1	23.5	25.6	32.0
11.	8.3	0.0	6.4	7.0	55.	0.0	0.0	0.0	0.0
12.	0.0	0.0	0.0	0.0	56.	0.0	0.0	0.0	0.0
13.	0.0	0.0	0.0	0.0	57.	0.0	0.0	0.0	0.0
14.	22.2	5.9	91.0	96.0	58.	0.0	0.0	1.3	2.0
15.	0.0	0.0	1.3	1.0	59.	2.8	2.9	6.4	7.0
16.	22.2	17.6	39.7	46.0	60.	61.1	52.9	60.3	58.0
17.	0.0	0.0	0.0	0.0	61.	0.0	0.0	2.6	4.0
18.	2.8	2.9	1.3	3.0	62.	2.8	2.9	24.4	13.0
19.	11.1	0.0	3.8	3.0	63.	5.6	2.9	20.5	13.0
20.	0.0	0.0	0.0	0.0	64.	0.0	0.0	0.0	0.0
21.	8.3	8.8	16.7	20.0	65.	0.0	0.0	0.0	0.0
22.	88.9	76.5	87.2	76.0	66.	41.7	50.0	21.8	26.0
23.	0.0	0.0	0.0	1.0	67.	55.6	67.6	60.3	70.0
24.	0.0	0.0	0.0	0.0	68.	19.4	26.5	17.9	33.0
25.	2.8	2.9	16.7	10.0	69.	0.0	0.0	2.6	1.0
26.	66.7	47.1	69.2	69.0	70.	0.0	2.9	1.3	1.0
27.	38.9	50.0	65.4	65.0	71.	0.0	2.9	5.1	1.0
28.	0.0	0.0	0.0	0.0	72.	19.4	17.6	6.4	8.0
29.	69.4	55.9	66.7	62.0	73.	97.2	91.2	98.7	96.0
30.	66.7	73.5	87.2	83.0	74.	25.0	26.5	30.8	24.0
31.	0.0	0.0	0.0	0.0	75.	2.8	5.9	50.0	57.0
32.	0.0	0.0	0.0	0.0	76.	66.7	38.2	35.9	33.0
33.	52.8	64.7	30.8	22.0	77.	8.3	2.9	5.1	1.0
34.	0.0	2.9	3.8	0.0	78.	0.0	0.0	0.0	5.0
35.	0.0	0.0	10.3	11.0	79.	55.6	50.0	75.6	62.0
36.	2.8	0.0	6.4	9.0	80.	5.6	0.0	15.4	13.0
37.	13.9	11.8	12.8	13.0	81.	50.0	58.8	79.5	65.0
38.	0.0	0.0	0.0	0.0	82.	94.4	85.3	87.2	95.0
39.	0.0	0.0	0.0	0.0	83.	2.8	2.9	6.4	7.0
40.	0.0	0.0	0.0	0.0	84.	0.0	11.8	3.8	2.0
41.	0.0	0.0	0.0	0.0	85.	0.0	0.0	0.0	0.0
42.	0.0	2.9	0.0	4.0	86.	2.8	0.0	67.9	42.0
43.	66.7	58.8	78.2	76.0	87.	41.7	41.2	39.7	63.0
44.	47.2	41.2	50.0	58.0	88.	5.6	5.9	20.5	20.0

Table D2. Frequency of occurrence of each skeletal variant in the first selected generation (G3) of a four-way outcross mouse population (C57BL, CBA, C3H, A = CBHA), selection being applied for susceptibility (S) and resistance (R) to trypan blue teratogenesis.

Character Number N=	3S 196	3R 56	3C 40	3U 65
1.	4.1	8.9	12.5	27.7
2.	15.8	19.6	15.0	9.2
3.	0.0	0.0	0.0	0.0
4.	0.0	0.0	0.0	0.0
5.	0.0	0.0	0.0	0.0
6.	3.6	3.6	5.0	3.1
7.	0.0	0.0	0.0	0.0
8.	44.9	44.6	45.0	52.3
9.	22.4	23.2	17.5	16.9
10.	22.4	17.9	30.0	30.8
11.	6.1	5.4	5.0	1.5
12.	0.0	0.0	0.0	0.0
13.	0.0	0.0	0.0	0.0
14.	94.9	96.4	65.0	12.3
15.	2.6	1.8	0.0	1.5
16.	60.7	37.5	42.5	13.8
17.	0.0	0.0	0.0	0.0
18.	2.0	3.6	0.0	0.0
19.	4.6	1.8	10.0	4.6
20.	0.5	0.0	0.0	0.0
21.	17.9	8.9	40.0	20.0
22.	91.8	91.1	75.0	95.4
23.	0.5	0.0	0.0	0.0
24.	0.0	0.0	0.0	0.0
25.	6.6	1.8	2.5	1.5
26.	75.0	64.3	57.5	56.9
27.	64.3	76.8	65.0	56.9
28.	0.0	0.0	0.0	0.0
29.	66.3	69.6	55.0	78.5
30.	80.6	94.6	75.0	73.8
31.	0.0	0.0	0.0	0.0
32.	0.0	0.0	0.0	0.0
33.	18.4	16.1	52.5	69.2
34.	2.6	3.6	2.5	0.0
35.	15.3	17.9	12.5	18.5
36.	4.6	1.8	0.0	1.5
37.	23.0	32.1	50.0	49.2
38.	0.0	0.0	0.0	0.0
39.	0.0	0.0	0.0	0.0
40.	0.0	0.0	0.0	0.0
41.	0.0	0.0	0.0	0.0
42.	7.1	5.4	2.5	3.1
43.	87.8	83.9	85.0	73.8
44.	61.7	76.8	52.5	61.5
45.	1.5	0.0	2.5	4.6
46.	2.6	1.8	5.0	0.0
47.	0.0	0.0	0.0	0.0
48.	0.5	1.8	5.0	0.0
49.	4.1	3.6	0.0	3.1
50.	0.0	0.0	0.0	0.0
51.	53.6	44.6	45.0	20.0
52.	9.7	7.1	7.5	18.5
53.	13.8	12.5	10.0	12.3
54.	39.8	53.6	37.5	26.2
55.	0.5	0.0	2.5	0.0
56.	0.0	0.0	0.0	0.0
57.	0.0	0.0	0.0	0.0
58.	0.0	0.0	0.0	0.0
59.	1.5	1.8	0.0	0.0
60.	65.8	66.1	65.0	72.3
61.	0.0	0.0	0.0	1.5
62.	9.2	10.7	5.0	3.1
63.	14.3	12.5	12.5	1.5
64.	0.0	1.8	0.0	1.5
65.	0.0	0.0	0.0	0.0
66.	6.1	8.9	12.5	27.7
67.	85.2	82.1	80.0	78.5
68.	8.7	8.9	7.5	3.1
69.	0.5	0.0	0.0	0.0
70.	1.0	0.0	0.0	0.0
71.	0.0	0.0	0.0	0.0
72.	8.2	7.1	30.0	20.0
73.	96.9	98.2	100.0	100.0
74.	19.9	37.5	25.0	46.2
75.	62.2	62.5	32.5	9.2
76.	30.6	39.3	57.5	55.4
77.	0.0	0.0	0.0	0.0
78.	1.5	0.0	0.0	0.0
79.	76.0	66.1	85.0	75.4
80.	0.0	0.0	0.0	0.0
81.	82.7	71.4	85.0	75.4
82.	87.2	89.3	90.0	89.2
83.	0.5	5.4	12.5	9.2
84.	1.0	0.0	2.5	0.0
85.	0.0	0.0	0.0	0.0
86.	56.1	46.4	37.5	9.2
87.	37.8	39.3	37.5	46.2
88.	10.7	1.8	10.0	1.5

Table D3. Frequency of occurrence of each skeletal variant in the sixth generation (G6) of CBHA four-way outcross mice, an untreated generation following three generations (G3-G5) of selection for susceptibility (S) and resistance (R) to trypan blue teratogenesis.

Character Number	6S	6SC	6RC	6U
N=	20	122	69	78
1.	0.0	2.5	1.4	0.0
2.	10.0	6.6	10.1	6.4
3.	0.0	0.0	0.0	0.0
4.	0.0	0.0	0.0	0.0
5.	0.0	0.0	0.0	0.0
6.	15.0	7.4	5.8	10.3
7.	0.0	0.0	0.0	0.0
8.	45.0	41.8	42.0	48.7
9.	20.0	15.6	27.5	14.1
10.	15.0	7.4	10.1	15.4
11.	30.0	8.2	0.0	3.8
12.	0.0	0.0	0.0	0.0
13.	0.0	0.0	0.0	0.0
14.	50.0	20.5	8.7	10.3
15.	0.0	0.8	0.0	0.0
16.	30.0	45.9	27.5	20.5
17.	0.0	1.6	0.0	0.0
18.	0.0	5.7	1.4	1.3
19.	0.0	2.5	10.1	5.1
20.	0.0	0.0	0.0	0.0
21.	15.0	18.0	21.7	11.5
22.	95.0	96.7	92.8	96.2
23.	0.0	0.0	1.4	0.0
24.	0.0	0.0	0.0	0.0
25.	10.0	9.8	1.4	3.8
26.	60.0	48.4	66.7	65.4
27.	70.0	39.3	46.4	60.3
28.	0.0	0.0	0.0	0.0
29.	85.0	82.0	76.8	82.1
30.	85.0	59.8	94.2	71.8
31.	0.0	0.0	0.0	0.0
32.	0.0	0.0	0.0	0.0
33.	65.0	81.1	68.1	87.2
34.	0.0	0.8	0.0	1.3
35.	15.0	18.9	15.9	12.8
36.	0.0	0.8	0.0	1.3
37.	60.0	51.6	52.2	37.2
38.	0.0	0.0	0.0	0.0
39.	0.0	0.0	0.0	0.0
40.	0.0	0.0	0.0	0.0
41.	0.0	0.0	0.0	0.0
42.	15.0	11.5	0.0	15.4
43.	75.0	64.8	81.2	88.5
44.	70.0	86.9	78.3	71.8
45.	0.0	0.8	1.4	7.7
46.	5.0	0.8	0.0	1.3
47.	0.0	0.0	0.0	0.0
48.	5.0	4.9	2.9	1.3
49.	5.0	4.9	1.4	1.3
50.	15.0	0.0	0.0	5.1
51.	35.0	25.4	26.1	34.6
52.	10.0	4.1	14.5	9.0
53.	25.0	7.4	17.4	6.4
54.	20.0	27.9	24.6	41.0
55.	5.0	0.0	0.0	0.0
56.	0.0	1.6	1.4	0.0
57.	0.0	0.0	0.0	0.0
58.	0.0	0.0	0.0	0.0
59.	0.0	0.0	0.0	2.6
60.	80.0	63.9	75.4	76.9
61.	0.0	0.0	0.0	0.0
62.	10.0	13.9	1.4	0.0
63.	5.0	11.5	1.4	1.3
64.	5.0	4.1	1.4	0.0
65.	0.0	0.0	0.0	0.0
66.	0.0	0.0	5.8	1.3
67.	75.0	65.6	56.5	79.5
68.	0.0	8.2	7.2	1.3
69.	0.0	0.0	0.0	0.0
70.	0.0	0.0	0.0	0.0
71.	0.0	0.0	0.0	0.0
72.	30.0	22.1	42.0	23.1
73.	100.0	96.7	100.0	100.0
74.	10.0	12.3	4.3	10.3
75.	40.0	12.3	36.2	9.0
76.	15.0	33.6	84.1	38.5
77.	0.0	0.0	0.0	0.0
78.	0.0	0.8	0.0	0.0
79.	85.0	82.8	72.5	93.6
80.	0.0	0.0	0.0	0.0
81.	85.0	83.6	75.4	93.6
82.	100.0	73.0	98.6	71.8
83.	0.0	0.0	2.9	1.3
84.	0.0	0.0	0.0	0.0
85.	0.0	0.0	0.0	0.0
86.	60.0	20.5	29.0	5.1
87.	40.0	52.5	63.8	32.1
88.	0.0	0.8	0.0	0.0

Table D4. Frequencies of occurrence of each skeletal variant in generation 7 of a four-way outcross mouse population (CBHA), with selection being resumed for susceptibility (S) and resistance (R) to trypan blue teratogenesis.

Character	7S	7R	7C	7U
Number N=	168	127	65	78
1.	8.3	5.5	7.7	2.6
2.	20.8	16.5	12.3	3.8
3.	0.0	0.0	0.0	0.0
4.	0.0	0.0	0.0	0.0
5.	0.0	0.0	0.0	0.0
6.	16.7	11.8	16.9	7.7
7.	0.0	0.0	0.0	0.0
8.	55.4	61.4	72.3	48.7
9.	9.5	22.8	6.2	5.1
10.	11.3	12.6	12.3	10.3
11.	7.1	4.7	4.6	2.6
12.	0.0	0.0	0.0	0.0
13.	0.0	0.0	0.0	0.0
14.	79.8	88.2	72.3	11.5
15.	4.2	0.8	0.0	0.0
16.	29.8	34.6	32.3	32.1
17.	0.6	0.0	0.0	3.8
18.	2.4	3.1	3.1	5.1
19.	1.8	0.8	0.0	0.0
20.	0.0	0.0	0.0	0.0
21.	9.5	5.5	18.5	20.5
22.	97.0	97.6	95.4	98.7
23.	0.0	3.1	0.0	1.3
24.	0.0	0.0	0.0	0.0
25.	7.1	14.2	4.6	2.6
26.	56.0	68.5	61.5	82.1
27.	50.6	64.6	76.9	62.8
28.	1.2	5.5	0.0	0.0
29.	85.1	76.4	84.6	85.9
30.	94.0	97.6	90.8	100.0
31.	0.0	0.0	0.0	0.0
32.	0.0	0.0	0.0	0.0
33.	40.5	19.7	38.5	65.4
34.	2.4	1.6	1.5	0.0
35.	28.0	32.3	24.6	11.5
36.	6.5	1.6	7.7	0.0
37.	58.9	63.8	64.6	59.0
38.	0.0	0.0	0.0	0.0
39.	0.0	0.0	0.0	0.0
40.	0.0	0.0	0.0	0.0
41.	0.0	0.0	0.0	0.0
42.	9.5	0.8	7.7	15.4
43.	73.2	95.3	89.2	82.1
44.	89.3	89.8	89.2	75.6
45.	5.4	1.6	15.4	19.2
46.	2.4	1.6	0.0	0.0
47.	0.6	0.0	1.5	0.0
48.	1.8	3.1	0.0	1.3
49.	1.8	0.8	1.5	1.3
50.	7.7	11.0	0.0	2.6
51.	3.0	5.5	4.6	1.3
52.	66.1	72.4	75.4	75.6
53.	10.7	23.6	6.2	9.0
54.	44.0	52.8	61.5	48.7
55.	0.0	0.0	0.0	1.3
56.	0.0	0.0	0.0	7.7
57.	0.0	0.0	0.0	0.0
58.	0.0	0.0	0.0	0.0
59.	2.4	1.6	1.5	0.0
60.	66.1	74.0	81.5	74.4
61.	0.0	0.0	0.0	1.3
62.	3.0	0.8	0.0	0.0
63.	23.2	16.5	13.8	2.6
64.	0.0	0.0	0.0	0.0
65.	0.0	0.0	0.0	0.0
66.	11.3	18.9	12.3	30.8
67.	85.7	92.1	89.2	94.9
68.	14.9	4.7	9.2	5.1
69.	0.0	0.0	3.1	1.3
70.	0.0	0.0	0.0	0.0
71.	1.2	8.7	7.7	1.3
72.	16.1	29.9	12.3	15.4
73.	92.9	98.4	96.9	94.9
74.	15.5	11.0	12.3	20.5
75.	51.2	82.7	61.5	16.7
76.	24.4	72.4	29.2	52.6
77.	0.6	6.3	1.5	1.3
78.	1.2	0.8	3.1	0.0
79.	91.7	96.9	90.8	94.9
80.	11.3	33.9	20.0	14.1
81.	86.3	95.3	90.8	87.2
82.	78.6	94.5	95.4	97.4
83.	4.8	10.2	3.1	0.0
84.	1.2	3.9	0.0	0.0
85.	0.0	0.0	0.0	0.0
86.	54.8	84.3	63.1	10.3
87.	42.3	52.8	16.9	25.6
88.	17.9	10.2	15.4	2.6

Table D5. Frequencies of occurrence of each skeletal variant in generation 9 of a four-way outcross mouse population (CBHA) having undergone six generations of selection (G3-G5, G7-G9) for susceptibility (S) and resistance (R) to trypan blue teratogenesis.

Character Number	9S	9R	9C	9U
N=	119	106	29	12
1.	5.9	2.6	3.4	8.3
2.	17.6	8.6	20.7	16.7
3.	0.0	0.0	0.0	0.0
4.	0.0	0.0	0.0	0.0
5.	0.0	0.0	0.0	0.0
6.	4.2	6.0	17.2	33.3
7.	0.0	0.0	0.0	0.0
8.	85.7	88.8	89.7	91.7
9.	2.5	2.6	0.0	0.0
10.	81.5	81.9	89.7	91.7
11.	0.0	0.0	0.0	0.0
12.	0.0	0.0	0.0	0.0
13.	0.0	0.0	0.0	0.0
14.	80.7	89.7	65.5	0.0
15.	5.0	0.0	0.0	0.0
16.	45.4	37.1	20.7	8.3
17.	0.8	0.0	0.0	0.0
18.	1.7	0.9	0.0	0.0
19.	1.7	0.9	0.0	0.0
20.	0.8	0.0	3.4	0.0
21.	10.9	6.0	6.9	8.3
22.	85.7	94.8	72.4	100.0
23.	0.0	0.0	0.0	0.0
24.	0.0	0.0	0.0	0.0
25.	2.5	1.7	0.0	0.0
26.	47.9	53.4	58.6	66.7
27.	48.7	52.6	79.3	75.0
28.	5.9	0.9	0.0	0.0
29.	78.2	85.3	96.6	100.0
30.	77.3	98.3	93.1	100.0
31.	0.0	0.0	0.0	0.0
32.	2.5	1.7	3.4	0.0
33.	17.6	15.5	17.2	41.7
34.	1.7	0.0	0.0	0.0
35.	59.7	75.0	82.8	41.7
36.	4.2	1.7	0.0	0.0
37.	71.4	81.0	62.1	66.7
38.	0.0	0.0	0.0	0.0
39.	0.0	0.0	0.0	0.0
40.	0.0	0.0	0.0	0.0
41.	0.0	0.0	0.0	0.0
42.	6.7	2.6	17.2	0.0
43.	71.4	93.1	79.3	75.0
44.	56.3	72.4	58.6	66.7
45.	0.8	0.0	20.7	16.7
46.	2.5	7.8	3.4	0.0
47.	0.0	1.7	0.0	0.0
48.	0.0	5.2	3.4	16.7
49.	1.7	1.7	0.0	0.0
50.	12.6	10.3	3.4	0.0
51.	8.4	11.2	0.0	0.0
52.	43.7	56.0	69.0	8.3
53.	6.7	9.5	0.0	0.0
54.	31.9	46.6	62.1	50.0
55.	0.0	0.0	0.0	0.0
56.	0.8	2.6	0.0	0.0
57.	0.8	0.0	0.0	0.0
58.	0.8	0.9	0.0	0.0
59.	0.0	2.6	6.9	0.0
60.	57.1	70.7	65.5	91.7
61.	0.0	0.0	0.0	0.0
62.	1.7	0.9	0.0	0.0
63.	9.2	13.8	10.3	0.0
64.	6.7	1.7	3.4	8.3
65.	0.8	0.0	0.0	0.0
66.	5.0	3.4	0.0	0.0
67.	81.5	86.2	93.1	83.3
68.	14.3	12.1	3.4	0.0
69.	0.0	0.9	3.4	0.0
70.	1.7	0.0	0.0	0.0
71.	2.5	0.0	0.0	0.0
72.	25.2	18.1	6.9	25.0
73.	77.3	99.1	96.6	100.0
74.	12.6	18.1	17.2	0.0
75.	68.1	94.0	51.7	0.0
76.	18.5	63.8	13.8	25.0
77.	0.8	6.9	0.0	0.0
78.	0.8	0.0	0.0	0.0
79.	80.7	98.3	100.0	75.0
80.	16.0	44.8	65.5	16.7
81.	83.2	99.1	100.0	75.0
82.	78.2	99.1	93.1	100.0
83.	5.0	2.6	0.0	0.0
84.	0.0	0.0	0.0	0.0
85.	0.0	0.0	0.0	0.0
86.	63.0	94.0	62.1	25.0
87.	44.5	36.2	31.0	8.3
88.	40.3	0.9	3.4	8.3

Table D6. Frequencies of occurrence of each skeletal variant in two rounds of treatment among selected lines in generation 9 of CBHA (combined values appear as columns 1 and 2 of Table D5).

Character Number	9S-I	9S-II	9R-I	9R-II
N=	57	62	76	40
1.	12.3	0.0	3.9	0.0
2.	22.8	12.9	9.2	7.5
3.	0.0	0.0	0.0	0.0
4.	0.0	0.0	0.0	0.0
5.	0.0	0.0	0.0	0.0
6.	5.3	3.2	3.9	10.0
7.	0.0	0.0	0.0	0.0
8.	100.0	72.6	88.2	90.0
9.	0.0	4.8	0.0	7.5
10.	94.7	69.4	80.3	85.0
11.	0.0	0.0	0.0	0.0
12.	0.0	0.0	0.0	0.0
13.	0.0	0.0	0.0	0.0
14.	84.2	77.4	85.5	97.5
15.	5.3	4.8	0.0	0.0
16.	47.4	43.5	30.3	50.0
17.	1.8	0.0	0.0	0.0
18.	1.8	1.6	1.3	0.0
19.	0.0	3.2	1.3	0.0
20.	0.0	1.6	0.0	0.0
21.	14.0	8.1	9.2	0.0
22.	98.2	74.2	94.7	95.0
23.	0.0	0.0	0.0	0.0
24.	0.0	0.0	0.0	0.0
25.	1.8	3.2	0.0	5.0
26.	50.9	45.2	57.9	45.0
27.	56.1	41.9	50.0	57.5
28.	1.8	9.7	0.0	2.5
29.	87.7	69.4	89.5	77.5
30.	100.0	56.5	100.0	95.0
31.	0.0	0.0	0.0	0.0
32.	0.0	4.8	2.6	0.0
33.	8.8	25.8	11.8	22.5
34.	0.0	3.2	0.0	0.0
35.	66.7	53.2	71.1	82.5
36.	0.0	8.1	0.0	5.0
37.	86.0	58.1	86.8	70.0
38.	0.0	0.0	0.0	0.0
39.	0.0	0.0	0.0	0.0
40.	0.0	0.0	0.0	0.0
41.	0.0	0.0	0.0	0.0
42.	8.8	4.8	3.9	0.0
43.	73.7	69.4	94.7	90.0
44.	70.2	43.5	68.4	80.0
45.	0.0	1.6	0.0	0.0
46.	1.8	3.2	5.3	12.5
47.	0.0	0.0	1.3	2.5
48.	0.0	0.0	6.6	2.5
49.	1.8	1.6	1.3	2.5
50.	26.3	0.0	11.8	7.5
51.	1.8	14.5	11.8	10.0
52.	61.4	27.4	52.6	62.5
53.	8.8	4.8	9.2	10.0
54.	42.1	22.6	52.6	35.0
55.	0.0	0.0	0.0	0.0
56.	0.0	1.6	1.3	5.0
57.	0.0	1.6	0.0	0.0
58.	0.0	1.6	1.3	0.0
59.	0.0	0.0	1.3	5.0
60.	70.2	45.2	76.3	60.0
61.	0.0	0.0	0.0	0.0
62.	0.0	3.2	1.3	0.0
63.	10.5	8.1	15.8	10.0
64.	10.5	3.2	2.6	0.0
65.	0.0	1.6	0.0	0.0
66.	0.0	9.7	0.0	10.0
67.	93.0	71.0	90.8	77.5
68.	14.0	14.5	10.5	15.0
69.	0.0	0.0	1.3	0.0
70.	0.0	3.2	0.0	0.0
71.	3.5	1.6	0.0	0.0
72.	26.3	24.2	18.4	17.5
73.	94.7	61.3	100.0	97.5
74.	8.8	16.1	25.0	5.0
75.	94.7	43.5	90.8	100.0
76.	22.8	14.5	63.2	65.0
77.	1.8	0.0	1.3	17.5
78.	1.8	0.0	0.0	0.0
79.	80.7	80.6	98.7	97.5
80.	12.3	19.4	43.4	47.5
81.	84.2	82.3	100.0	97.5
82.	94.7	62.9	98.7	100.0
83.	0.0	9.7	1.3	5.0
84.	0.0	0.0	0.0	0.0
85.	0.0	0.0	0.0	0.0
86.	75.4	51.6	96.1	90.0
87.	47.4	41.9	34.2	40.0
88.	40.4	40.3	13.2	15.0

Table D7. Frequency of each character among untreated, unselected mice (CBHA-C)

Char.	G3U	G6U	G7U	G9U	Average
N=	65	78	78	12	
1.	27.7	0.0	2.6	8.3	9.6
2.	9.2	6.4	3.8	16.7	9.0
3.	0.0	0.0	0.0	0.0	0.0
4.	0.0	0.0	0.0	0.0	0.0
5.	0.0	0.0	0.0	0.0	0.0
6.	3.1	10.3	7.7	33.3	13.6
7.	0.0	0.0	0.0	0.0	0.0
8.	52.3	48.7	48.7	91.7	60.4
9.	16.9	14.1	5.1	0.0	9.0
10.	30.8	15.4	10.3	91.7	37.0
11.	1.5	3.8	2.6	0.0	2.0
12.	0.0	0.0	0.0	0.0	0.0
13.	0.0	0.0	0.0	0.0	0.0
14.	12.3	10.3	11.5	0.0	8.5
15.	1.5	0.0	0.0	0.0	0.4
16.	13.8	20.5	32.1	8.3	18.7
17.	0.0	0.0	3.8	0.0	1.0
18.	0.0	1.3	5.1	0.0	1.6
19.	4.6	5.1	0.0	0.0	2.4
20.	0.0	0.0	0.0	0.0	0.0
21.	20.0	11.5	20.5	8.3	15.1
22.	95.4	96.2	98.7	100.0	97.6
23.	0.0	0.0	1.3	0.0	0.3
24.	0.0	0.0	0.0	0.0	0.0
25.	1.5	3.8	2.6	0.0	2.0
26.	56.9	65.4	82.1	66.7	67.8
27.	56.9	60.3	62.8	75.0	63.8
28.	0.0	0.0	0.0	0.0	0.0
29.	78.5	82.1	85.9	100.0	86.6
30.	73.8	71.8	100.0	100.0	86.4
31.	0.0	0.0	0.0	0.0	0.0
32.	0.0	0.0	0.0	0.0	0.0
33.	69.2	87.2	65.4	41.7	65.9
34.	0.0	1.3	0.0	0.0	0.3
35.	18.5	12.8	11.5	41.7	21.1
36.	1.5	1.3	0.0	0.0	0.7
37.	49.2	37.2	59.0	66.7	53.0
38.	0.0	0.0	0.0	0.0	0.0
39.	0.0	0.0	0.0	0.0	0.0
40.	0.0	0.0	0.0	0.0	0.0
41.	0.0	0.0	0.0	0.0	0.0
42.	3.1	15.4	15.4	0.0	8.5
43.	73.8	88.5	82.1	75.0	79.8
44.	61.5	71.8	75.6	66.7	68.9
45.	4.6	7.7	19.2	16.7	12.1
46.	0.0	1.3	0.0	0.0	0.3
47.	0.0	0.0	0.0	0.0	0.0
48.	0.0	1.3	1.3	16.7	4.8
49.	3.1	1.3	1.3	0.0	1.4
50.	0.0	5.1	2.6	0.0	1.9
51.	20.0	34.6	1.3	0.0	14.0
52.	18.5	9.0	75.6	8.3	27.9
53.	12.3	6.4	9.0	0.0	6.9
54.	26.2	41.0	48.7	50.0	41.5
55.	0.0	0.0	1.3	0.0	0.3
56.	0.0	0.0	7.7	0.0	1.9
57.	0.0	0.0	0.0	0.0	0.0
58.	0.0	0.0	0.0	0.0	0.0
59.	0.0	2.6	0.0	0.0	0.6
60.	72.3	76.9	74.4	91.7	78.8
61.	1.5	0.0	1.3	0.0	0.7
62.	3.1	0.0	0.0	0.0	0.8
63.	1.5	1.3	2.6	0.0	1.3
64.	1.5	0.0	0.0	8.3	2.5
65.	0.0	0.0	0.0	0.0	0.0
66.	27.7	1.3	30.8	0.0	14.9
67.	78.5	79.5	94.9	83.3	84.0
68.	3.1	1.3	5.1	0.0	2.4
69.	0.0	0.0	1.3	0.0	0.3
70.	0.0	0.0	0.0	0.0	0.0
71.	0.0	0.0	1.3	0.0	0.3
72.	20.0	23.1	15.4	25.0	20.9
73.	100.0	100.0	94.9	100.0	98.7
74.	46.2	10.3	20.5	0.0	19.2
75.	9.2	9.0	16.7	0.0	8.7
76.	55.4	38.5	52.6	25.0	42.9
77.	0.0	0.0	1.3	0.0	0.3
78.	0.0	0.0	0.0	0.0	0.0
79.	75.4	93.6	94.9	75.0	84.7
80.	0.0	0.0	14.1	16.7	7.7
81.	75.4	93.6	87.2	75.0	82.8
82.	89.2	71.8	97.4	100.0	89.6
83.	9.2	1.3	0.0	0.0	2.6
84.	0.0	0.0	0.0	0.0	0.0
85.	0.0	0.0	0.0	0.0	0.0
86.	9.2	5.1	10.3	25.0	12.4
87.	46.2	32.1	25.6	8.3	28.0
88.	1.5	0.0	2.6	8.3	3.1

Table D8. Frequency of each character among treated four-way outcross mice not selected for trypan blue response (CBHA-CT).

Char.	G3CT	G7CT	G9CT	Average	Char.	G3CT	G7CT	G9CT	Average
N=	40	65	29			40	65	29	
1.	12.5	7.7	3.4	7.9	45.	2.5	15.4	20.7	12.9
2.	15.0	12.3	20.7	16.0	46.	5.0	0.0	3.4	2.8
3.	0.0	0.0	0.0	0.0	47.	0.0	1.5	0.0	0.5
4.	0.0	0.0	0.0	0.0	48.	5.0	0.0	3.4	2.8
5.	0.0	0.0	0.0	0.0	49.	0.0	1.5	0.0	0.5
6.	5.0	16.9	17.2	13.1	50.	0.0	0.0	3.4	1.1
7.	0.0	0.0	0.0	0.0	51.	45.0	4.6	0.0	16.5
8.	45.0	72.3	89.7	69.0	52.	7.5	75.4	69.0	50.6
9.	17.5	6.2	0.0	7.9	53.	10.0	6.2	0.0	5.4
10.	30.0	12.3	89.7	44.0	54.	37.5	61.5	62.1	53.7
11.	5.0	4.6	0.0	3.2	55.	2.5	0.0	0.0	0.8
12.	0.0	0.0	0.0	0.0	56.	0.0	0.0	0.0	0.0
13.	0.0	0.0	0.0	0.0	57.	0.0	0.0	0.0	0.0
14.	65.0	72.3	65.5	67.6	58.	0.0	0.0	0.0	0.0
15.	0.0	0.0	0.0	0.0	59.	0.0	1.5	6.9	2.8
16.	42.5	32.3	20.7	31.8	60.	65.0	81.5	65.5	70.7
17.	0.0	0.0	0.0	0.0	61.	0.0	0.0	0.0	0.0
18.	0.0	3.1	0.0	1.0	62.	5.0	0.0	0.0	1.7
19.	10.0	0.0	0.0	3.3	63.	12.5	13.8	10.3	12.2
20.	0.0	0.0	3.4	1.1	64.	0.0	0.0	3.4	1.1
21.	40.0	18.5	6.9	21.8	65.	0.0	0.0	0.0	0.0
22.	75.0	95.4	72.4	80.9	66.	12.5	12.3	0.0	8.3
23.	0.0	0.0	0.0	0.0	67.	80.0	89.2	93.1	87.4
24.	0.0	0.0	0.0	0.0	68.	7.5	9.2	3.4	6.7
25.	2.5	4.6	0.0	2.4	69.	0.0	3.1	3.4	2.2
26.	57.5	61.5	58.6	59.2	70.	0.0	0.0	0.0	0.0
27.	65.0	76.9	79.3	73.7	71.	0.0	7.7	0.0	2.6
28.	0.0	0.0	0.0	0.0	72.	30.0	12.3	6.9	16.4
29.	55.0	84.6	96.6	78.7	73.	100.0	96.9	96.6	97.8
30.	75.0	90.8	93.1	86.3	74.	25.0	12.3	17.2	18.2
31.	0.0	0.0	0.0	0.0	75.	32.5	61.5	51.7	48.6
32.	0.0	0.0	3.4	1.1	76.	57.5	29.2	13.8	33.5
33.	52.5	38.5	17.2	36.1	77.	0.0	1.5	0.0	0.5
34.	2.5	1.5	0.0	1.3	78.	0.0	3.1	0.0	1.0
35.	12.5	24.6	82.8	40.0	79.	85.0	90.8	100.0	91.9
36.	0.0	7.7	0.0	2.6	80.	0.0	20.0	65.5	28.5
37.	50.0	64.6	62.1	58.9	81.	85.0	90.8	100.0	91.9
38.	0.0	0.0	0.0	0.0	82.	90.0	95.4	93.1	92.8
39.	0.0	0.0	0.0	0.0	83.	12.5	3.1	0.0	5.2
40.	0.0	0.0	0.0	0.0	84.	2.5	0.0	0.0	0.8
41.	0.0	0.0	0.0	0.0	85.	0.0	0.0	0.0	0.0
42.	2.5	7.7	17.2	9.1	86.	37.5	63.1	62.1	54.2
43.	85.0	89.2	79.3	84.5	87.	37.5	16.9	31.0	28.5
44.	52.5	89.2	58.6	66.8	88.	10.0	15.4	3.4	9.6

Table D9. Frequency of each character among four way outcross mice selected for resistance to trypan blue teratogenesis (CBHA-R)

Char.	G3R N= 56	G6RC 69	G7R 127	G9R 106	Average G3,7,9
1.	8.9	1.4	5.5	2.6	5.7
2.	19.6	10.1	16.5	8.6	14.9
3.	0.0	0.0	0.0	0.0	0.0
4.	0.0	0.0	0.0	0.0	0.0
5.	0.0	0.0	0.0	0.0	0.0
6.	3.6	5.8	11.8	6.0	7.1
7.	0.0	0.0	0.0	0.0	0.0
8.	44.6	42.0	61.4	88.8	65.0
9.	23.2	27.5	22.8	2.6	16.2
10.	17.9	10.1	12.6	81.9	37.5
11.	5.4	0.0	4.7	0.0	3.4
12.	0.0	0.0	0.0	0.0	0.0
13.	0.0	0.0	0.0	0.0	0.0
14.	96.4	8.7	88.2	89.7	91.4
15.	1.8	0.0	0.8	0.0	0.9
16.	37.5	27.5	34.6	37.1	36.4
17.	0.0	0.0	0.0	0.0	0.0
18.	3.6	1.4	3.1	0.9	2.5
19.	1.8	10.1	0.8	0.9	1.1
20.	0.0	0.0	0.0	0.0	0.0
21.	8.9	21.7	5.5	6.0	6.8
22.	91.1	92.8	97.6	94.8	94.5
23.	0.0	1.4	3.1	0.0	1.0
24.	0.0	0.0	0.0	0.0	0.0
25.	1.8	1.4	14.2	1.7	5.9
26.	64.3	66.7	68.5	53.4	62.1
27.	76.8	46.4	64.6	52.6	64.6
28.	0.0	0.0	5.5	0.9	2.1
29.	69.6	76.8	76.4	85.3	77.1
30.	94.6	94.2	97.6	98.3	96.9
31.	0.0	0.0	0.0	0.0	0.0
32.	0.0	0.0	0.0	1.7	0.6
33.	16.1	68.1	19.7	15.5	17.1
34.	3.6	0.0	1.6	0.0	1.7
35.	17.9	15.9	32.3	75.0	41.7
36.	1.8	0.0	1.6	1.7	1.7
37.	32.1	52.2	63.8	81.0	59.0
38.	0.0	0.0	0.0	0.0	0.0
39.	0.0	0.0	0.0	0.0	0.0
40.	0.0	0.0	0.0	0.0	0.0
41.	0.0	0.0	0.0	0.0	0.0
42.	5.4	0.0	0.8	2.6	2.9
43.	83.9	81.2	95.3	93.1	90.8
44.	76.8	78.3	89.8	72.4	79.7
45.	0.0	1.4	1.6	0.0	0.5
46.	1.8	0.0	1.6	7.8	3.7
47.	0.0	0.0	0.0	1.7	0.6
48.	1.8	2.9	3.1	5.2	3.4
49.	3.6	1.4	0.8	1.7	2.0
50.	0.0	0.0	11.0	10.3	7.1
51.	44.6	26.1	5.5	11.2	20.5
52.	7.1	14.5	72.4	56.0	45.2
53.	12.5	17.4	23.6	9.5	15.2
54.	53.6	24.6	52.8	46.6	51.0
55.	0.0	0.0	0.0	0.0	0.0
56.	0.0	1.4	0.0	2.6	0.9
57.	0.0	0.0	0.0	0.0	0.0
58.	0.0	0.0	0.0	0.9	0.3
59.	1.8	0.0	1.6	2.6	2.0
60.	66.1	75.4	74.0	70.7	70.3
61.	0.0	0.0	0.0	0.0	0.0
62.	10.7	1.4	0.8	0.9	4.1
63.	12.5	1.4	16.5	13.8	14.3
64.	1.8	1.4	0.0	1.7	1.2
65.	0.0	0.0	0.0	0.0	0.0
66.	8.9	5.8	18.9	3.4	10.4
67.	82.1	56.5	92.1	86.2	86.8
68.	8.9	7.2	4.7	12.1	8.6
69.	0.0	0.0	0.0	0.9	0.3
70.	0.0	0.0	0.0	0.0	0.0
71.	0.0	0.0	8.7	0.0	2.9
72.	7.1	42.0	29.9	18.1	18.4
73.	98.2	100.0	98.4	99.1	98.6
74.	37.5	4.3	11.0	18.1	22.2
75.	62.5	36.2	82.7	94.0	79.7
76.	39.3	84.1	72.4	63.8	58.5
77.	0.0	0.0	6.3	6.9	4.4
78.	0.0	0.0	0.8	0.0	0.3
79.	66.1	72.5	96.9	98.3	87.1
80.	0.0	0.0	33.9	44.8	26.2
81.	71.4	75.4	95.3	99.1	88.6
82.	89.3	98.6	94.5	99.1	94.3
83.	5.4	2.9	10.2	2.6	6.1
84.	0.0	0.0	3.9	0.0	1.3
85.	0.0	0.0	0.0	0.0	0.0
86.	46.4	29.0	84.3	94.0	74.9
87.	39.3	63.8	52.8	36.2	42.7
88.	1.8	0.0	10.2	0.9	4.3

Table D10. Frequency of each character among selected, susceptible mice (CBHA-S).

Char.	G3S N= 196	G6S 20	G7S 168	G9S 119	Average	Char.	G3S N= 196	G6S 20	G7S 168	G9S 119	Average
1.	4.1	0.0	8.3	5.9	4.6	45.	1.5	0.0	5.4	0.8	1.9
2.	15.8	10.0	20.8	17.6	16.1	46.	2.6	5.0	2.4	2.5	3.1
3.	0.0	0.0	0.0	0.0	0.0	47.	0.0	0.0	0.6	0.0	0.1
4.	0.0	0.0	0.0	0.0	0.0	48.	0.5	5.0	1.8	0.0	1.8
5.	0.0	0.0	0.0	0.0	0.0	49.	4.1	5.0	1.8	1.7	3.1
6.	3.6	15.0	16.7	4.2	9.9	50.	0.0	15.0	7.7	12.6	8.8
7.	0.0	0.0	0.0	0.0	0.0	51.	53.6	35.0	3.0	8.4	25.0
8.	44.9	45.0	55.4	85.7	57.7	52.	9.7	10.0	66.1	43.7	32.4
9.	22.4	20.0	9.5	2.5	13.6	53.	13.8	25.0	10.7	6.7	14.1
10.	22.4	15.0	11.3	81.5	32.6	54.	39.8	20.0	44.0	31.9	33.9
11.	6.1	30.0	7.1	0.0	10.8	55.	0.5	5.0	0.0	0.0	1.4
12.	0.0	0.0	0.0	0.0	0.0	56.	0.0	0.0	0.0	0.8	0.2
13.	0.0	0.0	0.0	0.0	0.0	57.	0.0	0.0	0.0	0.8	0.2
14.	94.9	50.0	79.8	80.7	76.3	58.	0.0	0.0	0.0	0.8	0.2
15.	2.6	0.0	4.2	5.0	2.9	59.	1.5	0.0	2.4	0.0	1.0
16.	60.7	30.0	29.8	45.4	41.5	60.	65.8	80.0	66.1	57.1	67.3
17.	0.0	0.0	0.6	0.8	0.4	61.	0.0	0.0	0.0	0.0	0.0
18.	2.0	0.0	2.4	1.7	1.5	62.	9.2	10.0	3.0	1.7	6.0
19.	4.6	0.0	1.8	1.7	2.0	63.	14.3	5.0	23.2	9.2	12.9
20.	0.5	0.0	0.0	0.8	0.3	64.	0.0	5.0	0.0	6.7	2.9
21.	17.9	15.0	9.5	10.9	13.3	65.	0.0	0.0	0.0	0.8	0.2
22.	91.8	95.0	97.0	85.7	92.4	66.	6.1	0.0	11.3	5.0	5.6
23.	0.5	0.0	0.0	0.0	0.1	67.	85.2	75.0	85.7	81.5	81.9
24.	0.0	0.0	0.0	0.0	0.0	68.	8.7	0.0	14.9	14.3	9.5
25.	6.6	10.0	7.1	2.5	6.6	69.	0.5	0.0	0.0	0.0	0.1
26.	75.0	60.0	56.0	47.9	59.7	70.	1.0	0.0	0.0	1.7	0.7
27.	64.3	70.0	50.6	48.7	58.4	71.	0.0	0.0	1.2	2.5	0.9
28.	0.0	0.0	1.2	5.9	1.8	72.	8.2	30.0	16.1	25.2	19.9
29.	66.3	85.0	85.1	78.2	78.6	73.	96.9	100.0	92.9	77.3	91.8
30.	80.6	85.0	94.0	77.3	84.2	74.	19.9	10.0	15.5	12.6	14.5
31.	0.0	0.0	0.0	0.0	0.0	75.	62.2	40.0	51.2	68.1	55.4
32.	0.0	0.0	0.0	2.5	0.6	76.	30.6	15.0	24.4	18.5	22.1
33.	18.4	65.0	40.5	17.6	35.4	77.	0.0	0.0	0.6	0.8	0.4
34.	2.6	0.0	2.4	1.7	1.7	78.	1.5	0.0	1.2	0.8	0.9
35.	15.3	15.0	28.0	59.7	29.5	79.	76.0	85.0	91.7	80.7	83.3
36.	4.6	0.0	6.5	4.2	3.8	80.	0.0	0.0	11.3	16.0	6.8
37.	23.0	60.0	58.9	71.4	53.3	81.	82.7	85.0	86.3	83.2	84.3
38.	0.0	0.0	0.0	0.0	0.0	82.	87.2	100.0	78.6	78.2	86.0
39.	0.0	0.0	0.0	0.0	0.0	83.	0.5	0.0	4.8	5.0	2.6
40.	0.0	0.0	0.0	0.0	0.0	84.	1.0	0.0	1.2	0.0	0.6
41.	0.0	0.0	0.0	0.0	0.0	85.	0.0	0.0	0.0	0.0	0.0
42.	7.1	15.0	9.5	6.7	9.6	86.	56.1	60.0	54.8	63.0	58.5
43.	87.8	75.0	73.2	71.4	76.8	87.	37.8	40.0	42.3	44.5	41.1
44.	61.7	70.0	89.3	56.3	69.3	88.	10.7	0.0	17.9	40.3	17.2

Table D11. Difference in frequency of each character between CBHA-S and CBHA-C.

Char.	N= Gen 3	Gen 6	Gen 7	Gen 9	Average $\bar{X}$	Average $\bar{X}$(Abso)
1.	-23.6	0.0	5.8	-2.5	-5.1	8.0
2.	6.6	3.6	17.0	1.0	7.0	7.0
3.	0.0	0.0	0.0	0.0	0.0	0.0
4.	0.0	0.0	0.0	0.0	0.0	0.0
5.	0.0	0.0	0.0	0.0	0.0	0.0
6.	0.5	4.7	9.0	-29.1	-3.7	10.8
7.	0.0	0.0	0.0	0.0	0.0	0.0
8.	-7.4	-3.7	6.6	-6.0	-2.6	5.9
9.	5.5	5.9	4.4	2.5	4.6	4.6
10.	-8.3	-0.4	1.1	-10.2	-4.5	5.0
11.	4.6	26.2	4.6	0.0	8.8	8.8
12.	0.0	0.0	0.0	0.0	-0.0	0.0
13.	0.0	0.0	0.0	0.0	0.0	0.0
14.	82.6	39.7	68.2	80.7	67.8	67.8
15.	1.0	0.0	4.2	5.0	2.6	2.6
16.	46.9	9.5	-2.3	37.0	22.8	23.9
17.	0.0	0.0	-3.3	0.8	-0.6	1.0
18.	2.0	-1.3	-2.7	1.7	-0.1	1.9
19.	-0.0	-5.1	1.8	1.7	-0.4	2.2
20.	0.5	0.0	0.0	0.8	0.3	0.3
21.	-2.1	3.5	-11.0	2.6	-1.8	4.8
22.	-3.5	-1.2	-1.7	-14.3	-5.2	5.2
23.	0.5	0.0	-1.3	0.0	-0.2	0.4
24.	0.0	0.0	0.0	0.0	0.0	0.0
25.	5.1	6.2	4.6	2.5	4.6	4.6
26.	18.1	-5.4	-26.1	-18.8	-8.0	17.1
27.	7.4	9.7	-12.2	-26.3	-5.3	13.9
28.	0.0	0.0	1.2	5.9	1.8	1.8
29.	-12.1	2.9	-0.8	-21.8	-8.0	9.4
30.	6.8	13.2	-6.0	-22.7	-2.2	12.2
31.	0.0	0.0	0.0	0.0	0.0	0.0
32.	0.0	0.0	0.0	2.5	0.6	0.6
33.	-50.9	-22.2	-24.9	-24.0	-30.5	30.5
34.	2.6	-1.3	2.4	1.7	1.3	2.0
35.	-3.2	2.2	16.4	18.0	8.4	9.9
36.	3.1	-1.3	6.5	4.2	3.1	3.8
37.	-26.3	22.8	-0.0	4.8	0.3	13.5
38.	0.0	0.0	0.0	0.0	0.0	0.0
39.	0.0	0.0	0.0	0.0	0.0	0.0
40.	0.0	0.0	0.0	0.0	0.0	0.0
41.	0.0	0.0	0.0	0.0	0.0	0.0
42.	4.1	-0.4	-5.9	6.7	1.1	4.3
43.	13.9	-13.5	-8.8	-3.6	-3.0	9.9
44.	0.2	-1.8	13.6	-10.4	0.4	6.5
45.	-3.1	-7.7	-13.9	-15.8	-10.1	10.1
46.	2.6	3.7	2.4	2.5	2.8	2.8
47.	0.0	0.0	0.6	0.0	0.1	0.1
48.	0.5	3.7	0.5	-16.7	-3.0	5.3
49.	1.0	3.7	0.5	1.7	1.7	1.7
50.	0.0	9.9	5.2	12.6	6.9	6.9
51.	33.6	0.4	1.7	8.4	11.0	11.0
52.	-8.8	1.0	-9.6	35.4	4.5	13.7
53.	1.5	18.6	1.7	6.7	7.1	7.1
54.	13.6	-21.0	-4.7	-18.1	-7.5	14.4
55.	0.5	5.0	-1.3	0.0	1.1	1.7
56.	0.0	0.0	-7.7	0.8	-1.7	2.1
57.	0.0	0.0	0.0	0.8	0.2	0.2
58.	0.0	0.0	0.0	0.8	0.2	0.2
59.	1.5	-2.6	2.4	0.0	0.3	1.6
60.	-6.5	3.1	-8.3	-34.5	-11.6	13.1
61.	-1.5	0.0	-1.3	0.0	-0.7	0.7
62.	6.1	10.0	3.0	1.7	5.2	5.2
63.	12.7	3.7	20.7	9.2	11.6	11.6
64.	-1.5	5.0	0.0	-1.6	0.5	2.0
65.	0.0	0.0	0.0	0.8	0.2	0.2
66.	-21.6	-1.3	-19.5	5.0	-9.3	11.8
67.	6.7	-4.5	-9.2	-1.8	-2.2	5.6
68.	5.6	-1.3	9.8	14.3	7.1	7.7
69.	0.5	0.0	-1.3	0.0	-0.2	0.4
70.	1.0	0.0	0.0	1.7	0.7	0.7
71.	0.0	0.0	-0.1	2.5	0.6	0.7
72.	-11.8	6.9	0.7	0.2	-1.0	4.9
73.	-3.1	0.0	-2.0	-22.7	-6.9	6.9
74.	-26.3	-0.3	-5.0	12.6	-4.7	11.0
75.	53.0	31.0	34.5	68.1	46.7	46.7
76.	-24.8	-23.5	-28.2	-6.5	-20.7	20.7
77.	0.0	0.0	-0.7	0.8	0.0	0.4
78.	1.5	0.0	1.2	0.8	0.9	0.9
79.	0.6	-8.6	-3.2	5.7	-1.4	4.5
80.	0.0	0.0	-2.8	-0.7	-0.9	0.9
81.	7.3	-8.6	-0.9	8.2	1.5	6.2
82.	-2.0	28.2	-18.9	-21.8	-3.6	17.7
83.	-8.7	-1.3	4.8	5.0	-0.0	5.0
84.	1.0	0.0	1.2	0.0	0.6	0.6
85.	0.0	0.0	0.0	0.0	0.0	0.0
86.	46.9	54.9	44.5	38.0	46.1	46.1
87.	-8.4	7.9	16.6	36.2	13.1	17.3
88.	9.2	0.0	15.3	32.0	14.1	14.1

Table D12. Difference in frequency of each character between CBHA-R and CBHA-C.

Char.	Gen 3	Gen 6 (RU)	Gen 7	Gen 9	Average $\bar{X}$	G 3,7,9 $\bar{X}$(Abso)
1.	-18.8	1.4	2.9	-5.7	-7.2	9.2
2.	10.4	3.7	12.7	-8.0	5.0	10.4
3.	0.0	0.0	0.0	0.0	0.0	0.0
4.	0.0	0.0	0.0	0.0	0.0	0.0
5.	0.0	0.0	0.0	0.0	0.0	0.0
6.	0.5	-4.5	4.1	-27.3	-7.6	10.6
7.	0.0	0.0	0.0	0.0	0.0	0.0
8.	-7.7	-6.7	12.7	-2.9	0.7	7.7
9.	6.3	13.4	17.7	2.6	8.9	8.9
10.	-12.9	-5.2	2.3	-9.8	-6.8	8.3
11.	3.8	-3.8	2.2	0.0	2.0	2.0
12.	0.0	0.0	0.0	0.0	0.0	0.0
13.	0.0	0.0	0.0	0.0	0.0	0.0
14.	84.1	-1.6	76.7	89.7	83.5	83.5
15.	0.2	0.0	0.8	0.0	0.3	0.3
16.	23.7	7.0	2.6	28.7	18.3	18.3
17.	0.0	0.0	-3.8	0.0	-1.3	1.3
18.	3.6	0.2	-2.0	0.9	0.8	2.1
19.	-2.8	5.0	0.8	0.9	-0.4	1.5
20.	0.0	0.0	0.0	0.0	0.0	0.0
21.	-11.1	10.2	-15.0	-2.3	-9.5	9.5
22.	-4.3	-3.4	-1.1	-5.2	-3.5	3.5
23.	0.0	1.4	1.9	0.0	0.6	0.6
24.	0.0	0.0	0.0	0.0	0.0	0.0
25.	0.2	-2.4	11.6	1.7	4.5	4.5
26.	7.4	1.3	-13.5	-13.2	-6.5	11.4
27.	19.9	-13.9	1.7	-22.4	-0.3	14.7
28.	0.0	0.0	5.5	0.9	2.1	2.1
29.	-8.8	-5.2	-9.5	-14.7	-11.0	11.0
30.	20.8	22.4	-2.4	-1.7	5.6	8.3
31.	0.0	0.0	0.0	0.0	0.0	0.0
32.	0.0	0.0	0.0	1.7	0.6	0.6
33.	-53.2	-19.1	-45.7	-26.1	-41.7	41.7
34.	3.6	-1.3	1.6	0.0	1.7	1.7
35.	-0.6	3.1	20.7	33.3	17.8	18.2
36.	0.2	-1.3	1.6	1.7	1.2	1.2
37.	-17.1	15.0	4.8	14.4	0.7	12.1
38.	0.0	0.0	0.0	0.0	0.0	0.0
39.	0.0	0.0	0.0	0.0	0.0	0.0
40.	0.0	0.0	0.0	0.0	0.0	0.0
41.	0.0	0.0	0.0	0.0	0.0	0.0
42.	2.3	-15.4	-14.6	2.6	-3.2	6.5
43.	10.1	-7.3	13.2	18.1	13.8	13.8
44.	15.2	6.5	14.1	5.7	11.7	11.7
45.	-4.6	-6.2	-17.7	-16.7	-13.0	13.0
46.	1.8	-1.3	1.6	7.8	3.7	3.7
47.	0.0	0.0	0.0	1.7	0.6	0.6
48.	1.8	1.6	1.9	-11.5	-2.6	5.0
49.	0.5	0.2	-0.5	1.7	0.6	0.9
50.	0.0	-5.1	8.5	10.3	6.3	6.3
51.	24.6	-8.5	4.2	11.2	13.4	13.4
52.	-11.3	5.5	-3.2	47.7	11.1	20.7
53.	0.2	11.0	14.6	9.5	8.1	8.1
54.	27.4	-16.4	4.0	-3.4	9.3	11.6
55.	0.0	0.0	-1.3	0.0	-0.4	0.4
56.	0.0	1.4	-7.7	2.6	-1.7	3.4
57.	0.0	0.0	0.0	0.0	0.0	0.0
58.	0.0	0.0	0.0	0.9	0.3	0.3
59.	1.8	-2.6	1.6	2.6	2.0	2.0
60.	-6.2	-1.6	-0.3	-21.0	-9.2	9.2
61.	-1.5	0.0	-1.3	0.0	-0.9	0.9
62.	7.6	1.4	0.8	0.9	3.1	3.1
63.	11.0	0.2	14.0	13.8	12.9	12.9
64.	0.2	1.4	0.0	-6.6	-2.1	2.3
65.	0.0	0.0	0.0	0.0	0.0	0.0
66.	-18.8	4.5	-11.9	3.4	-9.1	11.4
67.	3.7	-23.0	-2.7	2.9	1.3	3.1
68.	5.9	6.0	-0.4	12.1	5.8	6.1
69.	0.0	0.0	-1.3	0.9	-0.1	0.7
70.	0.0	0.0	0.0	0.0	0.0	0.0
71.	0.0	0.0	7.4	0.0	2.5	2.5
72.	-12.9	19.0	14.5	-6.9	-1.7	11.4
73.	-1.8	0.0	3.6	-0.9	0.3	2.1
74.	-8.7	-5.9	-9.5	18.1	-0.0	12.1
75.	53.3	27.3	66.0	94.0	71.1	71.1
76.	-16.1	45.6	19.9	38.8	14.2	24.9
77.	0.0	0.0	5.0	6.9	4.0	4.0
78.	0.0	0.0	0.8	0.0	0.3	0.3
79.	-9.3	-21.1	2.0	23.3	5.3	11.5
80.	0.0	0.0	19.8	28.2	16.0	16.0
81.	-4.0	-18.2	8.1	24.1	9.4	12.1
82.	0.1	26.8	-2.9	-0.9	-1.3	1.3
83.	-3.9	1.6	10.2	2.6	3.0	5.6
84.	0.0	0.0	3.9	0.0	1.3	1.3
85.	0.0	0.0	0.0	0.0	0.0	0.0
86.	37.2	23.9	74.0	69.0	60.1	60.1
87.	-6.9	31.7	27.1	27.9	16.0	20.6
88.	0.2	0.0	7.7	-7.5	0.1	5.1

Table D13. Difference in frequency of each character between treated (CBHA-CT) and untreated (CBHA-C) unselected mice.

Char.	Gen 3	Gen 7	Gen 9	Average $\bar{X}$	Average $\bar{X}$(Abso)
1.	-15.2	7.7	-4.9	-4.1	9.2
2.	5.8	12.3	4.0	7.4	7.4
3.	0.0	0.0	0.0	0.0	0.0
4.	0.0	0.0	0.0	0.0	0.0
5.	0.0	0.0	0.0	0.0	0.0
6.	1.9	16.8	-16.1	0.9	11.6
7.	0.0	0.0	0.0	0.0	0.0
8.	-7.3	71.8	-2.0	20.8	27.0
9.	0.6	6.1	0.0	2.2	2.2
10.	-0.8	12.2	-2.0	3.1	5.0
11.	3.5	4.6	0.0	2.7	2.7
12.	0.0	0.0	0.0	0.0	0.0
13.	0.0	0.0	0.0	0.0	0.0
14.	52.7	72.2	65.5	63.5	63.5
15.	-1.5	0.0	0.0	-0.5	0.5
16.	28.7	32.0	12.4	24.3	24.3
17.	0.0	-0.0	0.0	-0.0	0.0
18.	0.0	3.0	0.0	1.0	1.0
19.	5.4	0.0	0.0	1.8	1.8
20.	0.0	0.0	3.4	1.1	1.1
21.	20.0	18.3	-1.4	12.3	13.2
22.	-20.4	94.4	-27.6	15.5	47.5
23.	0.0	-0.0	0.0	-0.0	0.0
24.	0.0	0.0	0.0	0.0	0.0
25.	1.0	4.6	0.0	1.9	1.9
26.	0.6	60.7	-8.0	17.7	23.1
27.	8.1	76.3	4.3	29.6	29.6
28.	0.0	0.0	0.0	0.0	0.0
29.	-23.5	83.8	-3.4	18.9	36.9
30.	1.2	89.8	-6.9	28.0	32.6
31.	0.0	0.0	0.0	0.0	0.0
32.	0.0	0.0	3.4	1.1	1.1
33.	-16.7	37.8	-24.4	-1.1	26.3
34.	2.5	1.5	0.0	1.3	1.3
35.	-6.0	24.5	41.1	19.9	23.9
36.	-1.5	7.7	0.0	2.1	3.1
37.	0.8	64.0	-4.6	20.1	23.1
38.	0.0	0.0	0.0	0.0	0.0
39.	0.0	0.0	0.0	0.0	0.0
40.	0.0	0.0	0.0	0.0	0.0
41.	0.0	0.0	0.0	0.0	0.0
42.	-0.6	7.5	17.2	8.1	8.5
43.	11.2	88.4	4.3	34.6	34.6
44.	-9.0	88.5	-8.0	23.8	35.2
45.	-2.1	15.2	4.0	5.7	7.1
46.	5.0	0.0	3.4	2.8	2.8
47.	0.0	1.5	0.0	0.5	0.5
48.	5.0	-0.0	-13.2	-2.7	6.1
49.	-3.1	1.5	0.0	-0.5	1.5
50.	0.0	-0.0	3.4	1.1	1.2
51.	25.0	4.6	0.0	9.9	9.9
52.	-11.0	74.6	60.6	41.4	48.7
53.	-2.3	6.1	0.0	1.3	2.8
54.	11.3	61.1	12.1	28.2	28.2
55.	2.5	-0.0	0.0	0.8	0.8
56.	0.0	-0.1	0.0	-0.0	0.0
57.	0.0	0.0	0.0	0.0	0.0
58.	0.0	0.0	0.0	0.0	0.0
59.	0.0	1.5	6.9	2.8	2.8
60.	-7.3	80.8	-26.1	15.8	38.1
61.	-1.5	-0.0	0.0	-0.5	0.5
62.	1.9	0.0	0.0	0.6	0.6
63.	11.0	13.8	10.3	11.7	11.7
64.	-1.5	0.0	-4.9	-2.1	2.1
65.	0.0	0.0	0.0	0.0	0.0
66.	-15.2	12.0	0.0	-1.1	9.1
67.	1.5	88.3	9.8	33.2	33.2
68.	4.4	9.2	3.4	5.7	5.7
69.	0.0	3.1	3.4	2.2	2.2
70.	0.0	0.0	0.0	0.0	0.0
71.	0.0	7.7	0.0	2.6	2.6
72.	10.0	12.2	-18.1	1.4	13.4
73.	0.0	96.0	-3.4	30.8	33.1
74.	-21.2	12.1	17.2	2.7	16.8
75.	23.3	61.4	51.7	45.5	45.5
76.	2.1	28.7	-11.2	6.5	14.0
77.	0.0	1.5	0.0	0.5	0.5
78.	0.0	3.1	0.0	1.0	1.0
79.	9.6	89.8	25.0	41.5	41.5
80.	0.0	19.9	48.9	22.9	22.9
81.	9.6	89.9	25.0	41.5	41.5
82.	0.8	94.4	-6.9	29.4	34.0
83.	3.3	3.1	0.0	2.1	2.1
84.	2.5	0.0	0.0	0.8	0.8
85.	0.0	0.0	0.0	0.0	0.0
86.	28.3	63.0	37.1	42.8	42.8
87.	-8.7	16.7	22.7	10.2	16.0
88.	8.5	15.4	-4.9	6.3	9.6

Appendix E. Publications and Papers in Progress Presenting the Results of Findings from Skeletal Variant Assay Studies.

E1. Skeletal Variant Assay System (SVAS) for Detection of Biological Effects of Environmental Contaminants. Toxicology and Applied Pharmacology 37: 149 (Abst), 1976.

E2. Postnatal Detection of Prenatal Exposure to Herbicides in Mice, Using Normally Occurring Variations in Skeletal Development. Teratology 15: 15A (Abst), 1977.

E3. Concentration and Toxicity of Trifluralin in CD-1 Mice, Presented Intragastrically or Intraperitoneally. Bulletin of Environmental Contamination and Toxicology. 20: 554-560, 1978.

E4. Postnatal Effects on the CD-1 Mouse Skeleton of Prenatal Exposure to Trypan Blue. Teratology 21: 27A (Abst), 1980.

E5. Assessment of Adult Skeletons to Detect Prenatal Exposure to 2,4,5-T or Trifluralin in Mice. Teratology 23(1): in press, Feb. 1981.

E6. Postnatal Effects on the CD-1 Mouse Skeleton of Prenatal Exposure to Acetazolamide. Teratology 23: in press (Abst), 1981.

E7. Another Specific Teratogenic Effect of Acetazolamide in the Mouse?

in manuscript, for submission to the Journal of Experimental Embryology and Morphology. (describes a new variant, Frontal Extension, seen only in acetazolamide-treated animals)

E8. Postnatal Effects on the Mouse Skeleton of Prenatal Exposure to Trypan Blue

in manuscript, for submission to Teratology. (presents the results of both series of trypan blue studies)

E9. The extent of fetal ossification in the mouse in response to several teratogens at various dose levels

in preparation. (will present data on cervical and caudal ossification, ribs and sternebrae, and bones of the fore- and hind-paws)

E10. Postnatal effect on the mouse skeleton of prenatal exposure to acetazolamide.

in preparation. (will present the data summarized in E7)

E11. Cryptic skeletal variation in mice selected for differing responses to trypan blue teratogenesis.

in preparation. (will present results of studies in the CBHA four-way outcross mice)

In addition, data are available for a paper on the BUDR studies. Finally, it might be useful to present to the Teratology Society a poster paper on the negative results.

Copies of E1-E6 are included in this Appendix.

Toxicology and Applied Pharmacology
Volume 37, Number 1, July 1976

137. *Skeletal Variant Assay System (SVAS) for Detection of Biological Effects of Environmental Contaminants.* SIDNEY L. BECK, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina. (W. Durham)

More than sixty variations of the normal skeleton are known in the mouse (Gruneberg, H., *Pathology of Development*, 1963), with predictable frequencies of occurrence in strains, lines, crosses, and wild populations. These are subject to modification by teratogenic agents (Runner, M. R., *J. Nat. Cancer Inst.* **151**: 637, 1954; Beck, S. L., and Smith, N. J., *Teratology* **5**: 250). A method is being developed for testing a variety of compounds for biological effects in mammals following prenatal exposure, based on their ability to alter frequencies of occurrence of these variants. The pilot study consists of four experimental groups of CD-1 mice, exposed during Days 6-15 of gestation via intragastric maternal administration of 3.3 ml of corn oil/kg, containing: (1) 2,4,5-T at 100 mg/kg, (2) 2,4,5-T at 20 mg/kg, (3) Trifluralin at 1.0 g/kg, (4) vehicle control, and (5) an unhandled control group. Litters are born in isolation, enumerated, weighed, and examined for gross malformations. The same set of manipulations is performed at weaning. At 60 days postnatal the animals are killed, partially digested with 1% KOH, their skeletons stained with alizarin red S, cleared with glycerin, then benzyl alcohol, and examined under benzyl alcohol with a dissecting microscope for a battery of variants. The same specimens are subsequently digested completely with papain; disarticulated bones are picked, defatted in acetone, dried, and examined microscopically for a second battery of variants. Qualitative and quantitative assessment of a large number of compounds should be possible using this system, even in the absence of gross malformations. The SVAS will be described and preliminary data will be presented.

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BECK, S. L., Department of Biology, Wheaton College, Norton, MA 02766

Postnatal detection of prenatal exposure to herbicides in mice, using normally occurring variations in skeletal development.

Four groups of CD-1 mice were treated between days 6-15 of gestation via intragastric maternal intubation of 3.3ml corn oil/kg body weight containing (1) Trifluralin at 1.0/kg (TFL), (2) 245-T at 100mg/kg (245T-H) - a reported teratogenic dose, (3) 245-T at 20mg/kg (245T-L) - a reported no-effect dose, or (4) the vehicle alone (VEH). A fifth group was untreated (UNTD). Litters were born in isolation, examined perinatally and at weaning, and killed at 62±2 days. Specimens were processed by the alizarin method for examination of more than 40 normally occurring variations in skeletal development. Disarticulated skeletons were then prepared by the papain method and examined for a second battery of more than 30 variants. More than 90 specimens from at least 10 litters were examined in each group. Fifty eight characters showed variation. Differences from UNTD ($t>2.6$, $P<.01$, on arcsin-square root transformed frequencies) occurred as follows: 245T-H (positive control) = 14, VEH = 5, 245T-L (negative control) = 5, TFL = 7. Average differences among significant traits were: TFL = 21±3%, 245T-H = 19±3%, 245T-L = 15±.7%, VEH = 10±2%. Both 245T-H and TFL showed clustering of variants and a few dramatic differences in frequency. Both compounds could be detected based on number, magnitude, and spectrum of differences from UNTD. (These experiments were performed while the author was on an IPA assignment to the U.S. Environmental Protection Agency, in the Toxic Effects Branch of the Environmental Toxicology Division, at the Health Effects Research Laboratory, Research Triangle Park, NC)

Bull. Environm. Contam. Toxicol. 20,554-560 (1978)

Concentration and Toxicity of Trifluralin in CD-1 Mice, Presented Intragastrically or Intraperitoneally

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In considering factors which can influence toxicity in experimental administration of compounds, differences associated with routes of administration receive frequent attention; those which might be attributable to differences in the concentration at which any given dose might be presented tend to be considered less often. Further, such reports have generally dealt with aqueous solutions. The *parenteral* route is usually found to produce toxic effects at a lower dose and in a shorter time than *per ora* presentation. (See BOYD 1972, and CASARETT and DOULL 1975, for example, for detailed discussions).

The report which follows presents the results of experiments involving administration of the herbicide Trifluralin, dissolved in corn oil, delivered at different concentrations for each dose, either by intragastric intubation or intraperitoneal injection. The investigations produced several surprising results. These included the fact that. in *per ora* administration there were clear cut differences in toxicity such that for each dose which did cause death, there was an inverse relationship between toxicity and concentration. This relationship was not seen in the intraperitoneal treatment groups. Further, there was no apparent difference in LD-50 in the two routes, and the time to death was greater in the intraperitoneally treated animals.

MATERIALS AND METHODS

Random-bred female CD-1 mice were purchased from Charles River Breeding Laboratories (Wilmington, Ma.) at 25gm size. They were housed five animals per cage, provided with water and Wayne Blox Chow *ad libitum*. They were weighed at the start of the experiment. Body weight averaged 36.6gms (N=235) at the time of treatment. Each animal received a single dose of the test compound on a body weight basis, either through intragastric intubation or intraperitoneal injection. Each experimental group consisted of five animals which continued to be housed together. Toxicity was defined as the proportion of treated animals in each group which died within 14 days of treatment. Mortality was recorded daily and representative animals were autopsied. Surviving animals were weighed again at 24 hours, 7 days, and 14 days after treatment.

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Trifluralin is the Eli Lilly brand of α,α,α-Trifluoro-2,6-dinitro-N,N-di-N propyl-p-toluidine. Samples were weighed, melted by gentle heating and dissolved in reagent grade corn oil (Fisher Scientific Co.). For some experiments each dose was weighed and dissolved separately; for others only the highest concentration was weighed, lower concentrations being made up by dilution with the corn oil vehicle. The compound is bright orange in color, has a melting point of 48°C, is insoluble in water, highly soluble in acetone and in the corn oil vehicle used.

Four discrete intubation experiments were peformed and several series were repeated on three occasions. In all, there were 23 concentration-dose combinations, and the modal number of animals per combination was 15. The first two series presented constant volumes of intubate with varying amounts of Trifluralin providing doses of Trifluralin ranging from .375 - 3.0 gm/kg in one series (total volume 3.3 ml/kg) and 2.5 - 10.0 gm/kg in the second (total volume 11.7 ml/kg). Volumes of corn oil (as distinct from solute) were calculated for each different dose. Corn oil controls (3.3 and 11.7 ml/kg respectively) completed the design.

The third and fourth series presented constant volumes of corn oil with varying amounts of dissolved Trifluralin, resulting in varying total volumes of intubate. In one series, doses between 2.5 and 7.5 gm/kg were presented in 3.3 ml of corn oil/kg body weight. The range of intubate volumes was thus 4.5 - 9.1 ml/kg. The fourth series presented doses of .375 - 3.0 gm Trifluralin/kg in 8.33 ml corn oil/kg, resulting in intubate volumes which varied from 8.6 - 10.7 ml/kg.

The replications were of the latter type of experiment (i.e. constant volume of corn oil).

The intraperitoneal experiments consisted of two replications in which constant volumes of corn oil were injected, each containing different doses of Trifluralin. Seven volumes were used, between 3.3 and 23.3 ml/kg. The larger volumes were easily tolerated in the intraperitoneal route. The doses of Trifluralin varied between .375 and 10.0 gm/kg, with corn oil controls in all but two groups. Not all doses were presented in all volumes, but each dose was presented in at least four different concentrations.

RESULTS

Per Ora Route - No differences attributable to the different series could be detected, and the pooled data for mortality in response to treatment are presented in Table 1. At each dose-level which was presented in several different concentrations, mortality was higher in the groups receiving a larger volume of corn oil in the intubate. That is to say, the same dose of Trifluralin was more toxic in more dilute solutions. No mortality occurred at

TABLE 1.

Percent mortality in female CD-1 mice following a single gastric intubation of Trifluralin in corn oil

Volume of Corn Oil Intubated	Dose of Trifluralin (gm/kg Body Weight)								
(ml/kg body wt.)	10.0	7.5	5.0	3.0	2.5	1.5	0.75	0.375	0
< 3.0	-	-	-	(0,0) 0	-	(0) 0	(0) 0	(0) 0	-
3.3	(2) 40	(0,0,0) 0	(1,0,0) 6.7	(1,1,0) 13.3	(0,0,0) 0	(0,0,0) 0	-	-	(0) 0
6.7	-	(2) 40	-	-	-	-	-	-	-
8.3	-	(3,2,1) 40	(1,4,1) 40	(1,2,1) 26.7	(2,2,2) 40	(0,0,0) 0	(0,0,0) 0	(0) 0	-
10.0	-	-	-	-	(3,3,0) 40	(1,1,0) 13.3	-	-	(0,0) 0
11.7	-	-	-	-	-	-	-	-	(0) 0

Legend: Each number in parenthesis is the number killed per group of five animals which constitutes a single replicate.

doses below 1.5 gm/kg. Nearly all mortality occurred within 24 hours of treatment. The few animals which died one or two days later were noticeably unwell at 24 hours following treatment. Orange discoloration of urine and of the fur in the perineal area of treated animals suggested excretion of water soluble metabolites. EMMERSON and ANDERSON (1966) report at least ten colored metabolites of Trifluralin present in the urine of rats treated with the compound. Autopsy revealed orange discoloration of fat and intestine but not caecum. Slight orange-yellow discoloration of the inner surface of the skin and of the stomach was also seen. The discolored tissues suggest the deposition of the compound or lipid soluble metabolites. None of the other organs or tissues showed any grossly pathological signs or any discoloration.

There were no major differences in weight changes during the course of treatment, between different treatment groups or between replicates within each group. Indeed combined weights of all survivors (N=198) at 24 hours (36.9 gm), seven days (36.5 gm) and fourteen days (36.8 gm) were remarkably constant.

Intraperitoneal Route - The data for frequency of mortality are presented in Table 2. In all but one of the 34 dose-volume combinations (3 gm Trifluralin/kg in 23.3 ml/kg corn oil) there was good agreement between replicates. For this route of administration there was no relationship suggested between concentration and toxicity. Interpolation suggests an LD 50 of about 4.6 gm Trifluralin/kg body weight. Of the 124 animals which died in treatment, although the majority died within one day (N=54), or two days (N=43), there was considerable variation, some surviving to ten (N=1), eleven (N=4) or even twelve days (N=2) following treatment. The average survival times ($\overline{X}$ ± SD) of those which died was 2.47 ± 2.54 days. Death was generally more rapid at higher doses, (7.5 and 10.0 gm/kg) and within each of the higher doses, death was more rapid at lower concentrations.

In all 34 Trifluralin-treated and all seven corn-oil control groups, there was a weight loss among survivors at 24 hours after treatment. Three control and seven treated groups showed some recovery of body weight by one week after treatment; the remaining groups showed a further weight reduction. At termination of the experiment, 14 days after treatment, 17 groups showed some recovery of body weight; the others continued to lose weight. Continued weight loss was more pronounced at the higher doses at which there were a significant number of survivors (3.0 and 5.0 gm Trifluralin/kg body weight), regardless of the volume of corn oil in which the dose was presented.

DISCUSSION

Our data for toxicity of Trifluralin in the mouse, obtained from injection, are in agreement with published values for oral presentation (WORTH 1970); STECHER *et.al.* 1968, p. 1074). There

TABLE 2.

Percent mortality in female CD-1 mice following a single intraperitoneal injection of Trifluralin in corn oil

Volume of Corn Oil Injected (ml/kg body wt.)	Dose of Trifluralin (gm/kg Body Weight) 10.0	7.5	5.0	3.0	1.5	.75	.375	0
3.33	-	-	-	(0,0) 0	(0,0) 0	(0) 0	(0) 0	(0) 0
5.0	-	-	-	(1,0) 10	(0,0) 0	(0) 0	(0) 0	(0) 0
8.33	-	(3,4) 70	(1,0) 10	(0,0) 0	(0,0) 0	(0) 0	(0) 0	(0) 0
11.67	(5,5) 100	(5,5) 100	(3,5) 80	(1,0) 10	(0,2) 20	(0) 0	(0) 0	(0) 0
16.67	(5,5) 100	(4,4) 80	(3,2) 50	(0,0) 0	(0,0) 0	-	-	(0) 0
20.0	(5,5) 100	(5,5) 100	(5,3) 80	(1,0) 10	-	-	-	(0) 0
23.3	(4,5) 90	(5,5) 100	(3,5) 80	(5,0) 50	-	-	-	(0) 0
Average % Killed	97.5	90.0	60.0	11.4	4.0	0	0	0

Legend: Each number in parenthesis represents the number killed per group of five animals.

was no suggestion of enhanced toxicity via the *i.p.* route. It is difficult to estimate an LD 50 for *per ora* administration in our study since toxicity varied dramatically with concentration for any given dose. The phenomenon of increased toxicity with increased dilution has been reported for *per ora* administration of aqueous solutions by several authors for a variety of compounds (FERGUSEN 1962; MOORE *et.al.* 1960; BOYD *et.al.* 1969; BALAZS 1970). Explanations for this phenomenon include solvent toxicity (BOYD 1972), especially when very large volumes are presented (CONSTANTOPOULOS and BOYD 1968), and increase in rate of emptying of the stomach with enhanced intestinal absorption (MOORE *et.al.* 1968). Solvent effects of vegetable oils may include a laxative effect (CASARETT and DOULL 1975, p. 136), but observation of the fecal pellets of treated mice did not suggest any such solvent effect in the present study.

The major finding, of increased toxicity with increasing dilution in the *per ora* administration, could be attributed to enhanced absorption of the compound in larger vehicle volumes while it is transiently present in the gut. This interpretation is consistent with the failure to demonstrate this phenomenon in *i.p.* presentation, since in the latter case the compound continues to be present in the closed abdominal cavity, and rapid absorption is not as crucial for toxicity to become manifest. Other observations also support a somewhat slower, more prolonged absorption from the *i.p.* presentation. These include the greater time to death, and pronounced and prolonged weight loss following *i.p.* presentation but not *per ora* presentation. One might expect a slower uptake of material from an oil solvent in the peritoneal cavity relative to its rate in the gut. Thus all the data are consistent.

In addition to being an interesting demonstration of an inverse relationship between toxicity and concentration of a compound in an oil solvent, these data suggest that not only dose but also concentration should be reported for a meaningful assessment of toxicity of various compounds. Differences in concentration of intubate might well contribute to the wide variations in reported LD 50 for Trifluralin in rats, for example. These values vary from 500 mg/kg (CHRISTENSEN and LUGINBYHL 1974, p. 773), to 3,700 mg/kg (THOMPSON 1973, p. 48), to more than 10,000 mg/kg (WORTH 1970).

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TERATOLOGY 21:27A (1980)

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Post-natal effects on the CD-1 mouse skeleton of prenatal exposure to trypan blue.

Two sets of experiments were performed (I, II), each involving i.p. injection of 0, .037, .075, .15, or .3% trypan blue in .25 cc of .9% NaCl on days 7, 8, and 9 of gestation, skeletons being examined at 62±2 DPN for 88 normally-occurring all-or-none variants. Frequencies of each variant were contrasted with untreated and vehicle treated groups. Variation occurred in 66 and 58 of the characters in the two series. The number of variants which differed significantly ($P<.01$) from either untreated or vehicle controls was dose-related. At .3% there were 18 in I and 22 in II compared with untreated, 24 and 17 compared with vehicle. Agreement between the two series, while not exact, was good. Most variants were increased in frequency by the treatment. Variants which differed importantly in the two series were: presence of an Interfrontal bone in the skull, a group of cervical (C) vertebra variants including Dyssymphysis C2, Foramina Transversaria Imperfecta of both C1 and C2, Accessory Transverse Foramina of C3-C6, Dyssymphysis or Fusions among C3-C7, fusions both in the Carpals and Tarsals, reduction below 30 in number of Caudal vertebrae, and Caudal Fusions - frequently accompanied by tail kinks. Clearly the animals examined at 62 DPN were the survivors, and they showed few grossly visible signs of prenatal exposure to teratogenic doses of trypan blue. A detailed study of their skeletons, however, revealed the more subtle influences of this insult. Other known teratogens have produced similar results. Post-natal studies of selected mouse skeletal variants may be a useful screening procedure for teratogenicity testing.

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Assessment of Adult Skeletons to Detect Prenatal Exposure to 2,4,5-T or Trifluralin in Mice.[1]

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1 Graph
3 Tables
3 Plates

Abbreviated Title: Adult Skeletal Assessment of In Utero Treatment

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ABSTRACT

A group of 88 spontaneously-occurring variations of the skeleton were studied in adult CD-1 mice which had been exposed in utero to the herbicides 2,4,5-T or Trifluralin administered to their dams by gavage, in an attempt to detect subtle biological effects of these compounds even in the absence of gross malformations. Sixty-seven characters showed variation in this population. Of these, 19 were significantly different in frequency from untreated in mice having received a teratogenic dose of 2,4,5-T, with an average difference of 23.7%. Seventeen of them were increased in frequency. By contrast, only 3 traits differed from untreated among mice receiving a no-effect dose of 2,4,5-T. Twelve traits differed significantly from untreated in the Trifluralin treated group, the average difference was 18.8%, with ten of the traits being increased in frequency.

Two clusters of affected variants specific to the 2,4,5-T high dose group included frontal bone variants in the skull (presence of an Interfrontal bone and fusion of the frontals) and variants in the cervical vertebrae (imperfect foramina in the first and second cervicals, dyssymphysis of the second cervical, and a shift of the arch foramen from the fourth to the fifth cervical). Two other effects peculiar to the 2,4,5-T treated specimens were a loss of the prominent dorsal spine of the second thoracic vertebra and a reduction in the number of caudal vertebrae. The most obvious effects specific to the Trifluralin treatment were an increase in occurrence of 14 ribs, an undoubled Foramen Ovale, and the occurrence of Accessory Foramina in the cervical vertebrae. A striking increase in frequency of Parted Frontals was seen in both 2,4,5-T high dose and Trifluralin treated groups.

The Skeletal Variant Assay System may be useful as a postnatal screen for detecting prenatal exposure to potentially noxious substances.

INTRODUCTION

In recent years it has been widely recognized that prenatal exposure to possibly noxious chemical and physical agents may result in subtle effects on development which, while not producing gross malformations during fetal life, might nonetheless result in defects detectable postnatally. While a great deal of interest has been focused on behavioral parameters (see Hutchings '78, and Coyle *et al*. '76 for reviews), the nervous, genital, and immune systems have also been recognized (Tanimura '76, Spyker '76) as undergoing considerable development after birth and have become targets for detailed studies in postnatal teratologic assessment schemes. The report which follows is a postnatal assessment scheme based on yet another system which undergoes considerable development after birth: the skeleton.

There exist in a number of mammalian species, a series of normally occurring discontinuous variations in development of the skeleton, primarily epigenetic in nature (Berry and Searle '63). Some of these have been described in the human (Le Double '03, '12, Cave '30, Comas '60, Brothwell '63), but most investigations of epigenetic polymorphism in skeletal development have used various rodent species (Berry and Searle '63) and the rabbit (Sawin '45, '46). The species most extensively studied has been the laboratory mouse, *Mus musculus* L. More than fifty such qualitative variations have been described by Grüneberg ('50, '51, '52, and later) and his collaborators (particularly Berry '63, Deol '55, Deol and Truslove '57, Deol *et al*. '57, Searle '54a,b,c, Truslove '52, '54, and Weber '50). Descriptions for most of these may be found in Berry and Searle's ('63) and Berry's ('63) papers. All told, about a hundred such traits have been described in the mouse. Eighty-eight characters were selected for the present study, including some not previously described or not described in detail.

Many of these variations exist as polymorphisms in laboratory strains (Deol et al., '57, Searle '54a) and in wild mice (Berry '63, Deol '58, Harland '58, Weber '50). The frequencies of occurrence of many of these variants are strain-specific and population specific (Berry '63). Thus, even though the presence or absence of a variant in a given animal appears to be epigenetically determined, consistent differences in frequencies among strains or populations reveal underlying genetic determinants of thresholds of appearance.

Soon after descriptions of a number of these variants appeared, Runner ('54) demonstrated that frequencies of occurrence of several of them could be influenced by a variety of teratogenic agents. Palmer ('72) also showed influence of drug administration on some of these. Beck and Smith ('72) and Beck ('73) showed that frequencies of a number of these skeletal variants were altered in populations of mice selected of susceptibility and resistance to trypan blue teratogenesis (Beck '71), relative to unselected animals from the same foundation population. The report which follows presents the results of the first series of experiments designed to detect prenatal exposure to environmental contaminants, in the absence of gross malformations, using alterations of frequency of occurrence among a large number of these spontaneous polymorphisms in skeletal development, assessed at two months after birth. Both positive and negative control groups are presented in addition to a compound of interest whose teratogenic properties are not fully known. The positive control is the herbicide 2,4,5-Trichlorophenoxyacetic acid (2,4,5-T) at a dose reported to be teratogenic in mice (Courtney et al. '70); the negative control is the same compound at a dose reported as non-teratogenic (Roll '71, Neubert and Dillman '72). The "unknown" compound being tested is Trifluralin, the Eli Lilly brand of α,α,α-Trifluoro-2,6-dinitro-N,N-di-N propyl-p-toluidine, which is a pre-emergence herbicide (Alder *et al*. '61, Ford '65) used extensively for control of a wide variety of grasses and broadleaf weeds (Leitis and Crosby '74). To complete the design for the present study, and to eliminate the possibility of solvent effects (Boyd '72) or maternal handling effects (Beck and Gavin '76) influencing the results, solvent controls and unhandled controls have also been included.

MATERIALS AND METHODS

Animals and Mating

CD-1 mice purchased from Charles River Breeding Laboratories (Wilm., Ma.) were provided with Wayne Blox Chow and water *ad libitum*. During an extended period of isolation animals were housed five of one sex per standard shoe-box cage on pine wood shavings. Each group of five females was introduced to a male, housed alone, at about 4:00 PM. The following morning at about 8:00 AM the mated females were identified as those having vaginal plugs. They were distributed to experimental pens. Five females, each from a different mating, comprised a single experimental pen, and received the same treatment. The order of experimental pens was randomized on the animal room shelves, and the females mated by a given male were distributed across experiments.

Agent Administration and Experimental Design

The treatments, described below, were presented by gavage on each of days 6-15 of pregnancy. The date the vaginal plug was detected was considered to be day zero of gestation. The intubated volume was 3.3 ml per kg. body weight per day of treatment. Experimental females were weighed to the nearest tenth of a gram just prior to the initial treatment. The dose administered to each mouse was based on the day 6 weight. Five groups were established:

Group I (TFL) received doses of 1.0 gm of Trifluralin (Lilly) per kg. body weight, per treatment, dissolved in reagent grade Corn Oil (Fisher).

Group II (245T-H) animals were dosed with 100 mg/kg/treatment of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) containing less than 0.5 ppm TCDD (Dow), dissolved in Corn Oil.

Group III (245T-L) animals received 20 mg/kg/treatment of 2,4,5-T in Corn Oil.

Group IV (VEH) animals received 3.3 ml/kg/treatment of Corn Oil Vehicle.

Group V (UNTD) animals were not treated or subjected to any handling other than routine animal room maintenance.

Litters were born in isolation, examined for gross malformations and weighed, soon after birth. Litters were weaned at 28 days after birth, sexed, examined again for malformations, and the sexes were weighed separately. Each litter continued to be housed as a unit, and animals were sacrificed at 62±2 days after birth.

Specimen Preparation and Observation

Following sacrifice, animals were skinned, eviscerated, numbered, immersed in 1% potassium hydroxide (KOH) for digestion of muscle. Alizarin Red-S, in a saturated 95% ethanol solution, was added to fresh KOH for staining of bones. The KOH was then replaced with 50% aqueous glycerin, two changes of pure glycerin and one or two changes of Benzyl Alcohol. Each specimen was examined submerged in Benzyl Alcohol, trans-illuminated, and with the aid of a

Bausch and Lomb SKV1070P stereoscopic microscope equipped with a 0.5X supplementary lens. Specimens were photographed with a Polaroid camera fitted to the microscope, or with a Nikon F2 Photomic camera with a 55 mm f3.5 Micro-P lens, with a green filter, with or without a bellows attachment. The 35 mm film was Kodak Plus-X and development was routine.

After examination of the articulated skeletons, specimens were boiled in tapwater for five minutes then individually incubated overnight or longer at 37°C in 75cc of a 0.9% NaCl solution to which was added approximately 25 mg of powdered Papain (Sigma). The papain solution was then decanted, and the disarticulated skeletons rinsed with tap water. The bones were covered with 100cc of acetone for four hours to defat and dehydrate. The acetone was decanted, the bones air dried, then carefully tipped into a glass petri dish and examined with the stereomicroscope, using reflected light. Photographs, when appropriate were made as above.

Parameters

Each articulated skeleton was examined for the battery of variants designated in Table 2, Column 1 with an "A" following the character number. The same skeleton, after disarticulation, were examined for the battery of variants designated in Table 2, Column 1 with a "D" following the character number. Descriptive nomenclature was that of Bateman('54). Diagrams, descriptions and references for most of the variants appear in the article by Berry and Searle ('63). Three variants observed in the present study which have not previously been described are:

19A. Frontal Foramen Absent (Figure 5). This foramen on the lateral surface of the Frontal bone near the dorsal edge of the orbit has been described as being doubled (i.e. two or more foramina in close proximity instead of a single foramen), but in the present study it has occasionally been absent as well.

20A. Accessory Parietal (Figure 4). A suture may delineate a separate section of the dorsal surface of either or both parietal bones in the anterior-medial region.

64A. Jointed Processus Spinosus of the Second Thoracic Vertebra (Figure 14).

This prominent dorsal spine may be divided by a horizontal unossified gap into two portions, the distal third being discontinuous with the remainder. Rarely, the distal third consists of two bones separated by a vertical gap. The disconnected portion is in the proper location, but gives the appearance of a joint in this vertebral appendage. The discontinuous portion would be unidentifiable in papain preparation, giving the appearance of a reduced spinous process.

In addition, two variants usually described in connection with studies of skeletal mutants (Grüneberg '63) are worth mentioning. These are:

75A. Carpal Fusions (Figure 18). Typically the second, but rarely the third, carpal may be fused with the centrale (Deol '61, Beck and Smith '72). The fourth and fifth carpals are invariably fused in the mouse.

86A. Fusion of Tarsals (Figure 17). Like carpal fusion this generally involves the second tarsal bone fused with the centrale; sometimes the second and third tarsals are fused with one another. (Grüneberg, '56, Deol '61, Beck and Smith '72)

All of the variants observed were scored as present or absent, although for many of them, manifestation did vary in extent or severity. The variants are thus classic examples of quasi-continuous characters (Grüneberg '52). Bilaterally expressed variants were classed as occurring if they were manifested on at least one side. Similarly, where a variant could occur at several locations (viz: Reduced Articular Processes of Thoracic Vertebrae), it was classed as present if it appeared in at least one location. Thus it is the number of individuals, not the number of sites, showing a trait which appear in the tables and figure. Dyssymphysis in a vertebra is defined as obvious signs of mismatching, with or without a gap, as a consequence of failure of ossification masses to meet and fuse properly. Most dyssymphysis in the vertebral column occurred in the neural arch. An animal was scored as having a fourteenth rib if it showed the presence of a rudimentary or complete rib on either or both sides. Marked asymmetries were detected (Figure 16). Most fusions detected in articulated specimens were verified in disarticulated skeletons.

Data Analysis

Frequencies were calculated for each variant, for each experimental group on the basis of the individual as the unit, and as unweighted averages of litter percents.

Chi-square tests were performed, and for those variants which were significantly different from UNTD ($P<.05$) in each experimental group, Mann-Whitney U tests (Siegal '56) were performed as were t-tests on arcsin-square root transformed frequencies (Sokal and Rohlf '69), with significance being defined as $P<.01$ to compensate for possible Type I errors due to litter effects. Initial comparisons were made between sexes among UNTD, and, finding few differences, the data were pooled across sexes. A comparison between each compound-treated group and the vehicle control was also performed for each variant. A divergence estimate using the method of C.A.B. Smith as described by Grewal ('62) and Berry ('63, '64) was calculated for each group as an index of differences from the untreated group. Several other simple manipulations of the data were performed but need not be separately described.

RESULTS

Initially 25 females were assigned to each treatment group. Because of maternal mortality in the high dose 2,4,5-T treated females (245T-H) during treatment, additional females were placed in this treatment. The results of treatment (Table 1) were surprising in that the dose of 2,4,5-T employed was one which had been reported as teratogenic, but not particularly toxic (Courtney *et al*. '70). A lesser amount of maternal mortality occurred in the other herbicide groups as well. Most, though not all, females which died were autopsied. Where death was attributable to intubation induced injury females were removed from consideration. Failure to detect intubation injury may inflate some of the maternal mortality values.

Some surviving females failed to produce litters in all groups. Occasional litters in the herbicide treated groups were stillborn. Even in live born litters, there were significantly higher proportions of stillborn pups in 245T-L, and especially in TFL, relative to Untreated controls. If one defines Mating Efficiency as the proportion of mated females which produced live litters, this parameter is seen to be reduced in all four treated groups relative to Untreated. Average litter birth weight relative to untreated, was significantly reduced only in the 245T-H group ($t=2.53$, $P<.05$, $N_1=23$ litters, $N_2=8$ litters). Litter sizes at birth were not significantly different in the different groups.

Some possibly abnormal young were produced in all groups, but a substantially greater number of peculiar neonates were seen in the TFL group than in the others. The frequency of atypical pups is highly significant in the TFL compared with untreated; for the vehicle, 245T-H, and 245T-L, the differences from untreated are of borderline significance. The peculiar pups included runts, seen in all groups, cases of mild hemorrhaging on the snout, tail, or feet, and possible tail kinks seen in 245T-L, Trifluralin, and vehicle control pups. Six pups from Trifluralin treated mothers and one each in 245T-H and 245T-L showed little or no movement of the hind limbs, which were crossed in the midline. These pups appeared to have narrow pelvices, and were unable to right themselves when turned on their backs. These pups died within 24 hours of birth, as did several of the runts.

Of the 88 skeletal variants examined in 62±2 day old animals whose dams had been in the experiment, 67 showed variation and are so labeled in Table 2. The Mann-Whitney U analysis, based on the litter as the experimental unit, indicated no significant differences in the negative control (245T-L) and only one, #47-Reduction of Spinous Process of Th2 (see Figure 13) in the vehicle control. The positive control group (245T-H) differs from untreated for nine variants, all but one at $P<.01$; and the Trifluralin treated group differs from untreated for seven variants, all at $P<.05$. There are clusters of frontal bone variants, cervical vertebra variants and the Reduction of the Spinous Process of Th2 which are revealed as strongly different from untreated in the 245T-H treated litters;

a thoracic cluster and a sprinkling of skull variants appear as affected in the Trifluralin treated group. Examining clusters of variants in the less conservative but perhaps more sensitive t-test analysis reveals, in 245T-H, #7-Interfrontal (Figure 2), #9-Parted Frontals (Figure 3), #10-Fused Frontals, #11-Frontal Foramen Double (Figure 5) in the frontal bone cluster, and among the cervical (C) vertebra variants: #33-dyssymphysis of C2 (Figure 9), #36-Absent Arch Foramina of C4 (Figures 7, 9), #37-Presence of Arch Foramina in C5 (Figure 7), Foramina Transversaria Imperfecta in C1 (#32, see Figure 8) and C2 (#34, see Figures 10 and 11) each considered separately, and C3-6 considered as a group (#38, see Figure 12), as well as the suggestion of a cluster of variants involving the spinous process of the second thoracic vertebra (Th2) including #47-Reduction or #46-Absence (Figure 15) of that prominent dorsal spine. In the TFL group the only suggestion of clustering appears in the thoracic region and involves increases in occurrence of Reduced Spinous Process of Th2 (#47), #52-Reduced Articular Processes of the Thoracic Vertebrae, and increased occurrence of #54-Fourteen Ribs (Figure 16).

The average absolute percent difference from Untreated of the variants which differ ($P<.01$) is 23.7±3.1 in the 245T-H group (N=19 variants), 16.1±1.1 in 245T-L (N=3), 15.1±2.1 in VEH (N=6), and 18.8±2.5 in TFL (N=12). The average percent difference from Untreated among significantly affected traits, taking direction of difference into account, is 5.31±6.2 for VEH, 4.83±10.9 for 245T-L, 14.86±4.6 for 245T-H, and 13.74±3.5 for TFL. Thus there is a slight overall increase in frequency of occurrence of these traits in VEH and 245T-L and a significant increase in 245T-H and TFL. For all 67 variants, and considering the direction of the differences, the average difference from Untreated is 0.19% for VEH, 0.14% for 245T-L, 5.69% for 245T-H, and 2.96% for TFL, suggesting that variation around Untreated values is nearly random for 245T-L and VEH.

The magnitude of the differences between frequency of occurrence of each trait in each experimental group from Untreated (experimental minus Untreated) are presented for each group in Figure 1. Those differences which are significant are represented by heavy bars, the others by light bars. By applying a simple sign test (Siegal '56) to each of the two positive groups, it is clear that significantly more of the affected variants show an increased frequency of occurrence relative to controls in both the TFL (N=11, P=.012) and 245T-H (N=18, P<.001) than show a decrease in response to treatment. Overall, the number of traits which were increased and decreased in frequency in the experimentals compared with untreated were, respectively, 22 and 17 in VEH, 24 and 16 in 245T-L, 36 and 11 in 245T-H, and 32 and 14 in TFL.

Correlations between dose and frequencies for 2,4,5-T treatments (0=vehicle, 20, 100 mg/kg) nominally occur in 17 of the 67 variants (#14,15,18,23,24,28,34,36,37,40,46,53,55,58,63,65,66). Of these, most are trivial. Among the 19 variants which are significantly affected in 245T-H, six suggest a dose related response (#34,36,37,46,63,66).

Several variants are significantly affected in both positive groups (#6,9,19,47,66). All but one (#66) are also significantly affected in the VEH group however; only Parted Frontals (#9) of this group of variants remain significantly affected in both positive groups when contrasted with the frequency in the Vehicle group. The Absence of the Arch Foramen in the Fourth Cervical vertebra (#36) becomes significantly different in both positive groups contrasted with Vehicle.

There are a number of variants which appear to be strongly affected in 245T-H and one in TFL experimental groups; these may be sensitive indicators for that particular treatment. For the 245T-H group they include #10,32,33,34,36,46,66; and in the TFL group, #54.

Those variants which differ both from UNTD and VEH frequencies could be said to be importantly affected by the compound treatments. They are: 245T-L = Foramina Transversaria Imperfecta C2 (#34) and Arch Foramen C4 Absent (#36); 245T-H = Preorbital Foramen Double (#1 - see Figure 5), Interfrontal (#7), Parted Frontals (#9), Fused Frontals (#10), Frontal Foramen Double (#11), Posterior Border of the Palatine-Anteriad Suture (#22), Foramina Transversaria Imperfecta of C1 (#32) and C2 (#34), Dyssymphysis C2 (#33) Arch Foramen C4 Absent (#36), Arch Foramen C5 Present (#37), Spinous Process Th2 Absent (#46), Sacral Fusions-Dorsal Processes (#60), and Fewer than 30 Caudal Vertebrae (#66); TFL = Parted Frontals (#9), Foramen Palatinum Minus Posterior Double (#17) and Absent (#18), Foramen Ovale Single (#23), Accessory Transverse Foramina C3-C6 (#39), and Fourteen Ribs (#54).

DISCUSSION

The study reported here was a first attempt at devising an objective and reproducible method of postnatal assessment of prenatal exposure to environmental contaminants in the absence of gross morphological abnormalities by systematically studying skeletal variation in adult, fully ossified, skeletons. Earlier teratologic investigations of normally occurring skeletal variants in adult mice (Runner '54, Beck and Smith '72) had different protocols and different purposes. Most of these variants have very little correlation with one another (Truslove '61) and so are independent parameters.

Examination at 60 days post natal allows considerable time for amplification of subtle effects through a developmental "Pedigree of Causes" (Grüneberg '38) without confounding age-related variations in development. There are of course a number of problems associated with examination of parameters the occurrence of which may be influenced by diet (Searle '54c, Deol and Truslove '57), maternal uterine environment (McLaren and Michie '58), and "inert" vehicles (present study, Gibson *et al*. '66, Kimmel and Wilson '73) as well as the known teratogens contained in those vehicles (Runner '54, Gibson '66, Beck and Smith, '72, Yasuda and Maeda '72, Kimmel and Wilson '73). By randomly assigning a single lot of commercially-bred animals of the same age across treatments in a reasonably well controlled experimental environment, it was felt that most of the extrinsic variables could be controlled.

One variable, litter effects, presented a more thorny problem. Without detailing the discussion of litter or individual mouse as an appropriate statistical unit (Weil '70, '74; Healy '72; Kalter '74; Staples and Haseman '74; Becker '74; Palmer '74; Haseman and Hogan '75), suffice it to say that frequencies were calculated for each parameter in each group both as mean litter percents and as number affected per total animals. Haseman and Hogan ('75) suggest that it might be preferable to use per-litter tests with a less conservative level of probability to indicate significance (e.g., 0.10 or 0.20 instead of the traditional 0.05 and 0.01) as a way of reducing "false negatives", rather than using per-fetus tests (per-individual in these experiments since the animals are examined postnatally). I have elected to take the other route; namely, to minimize "false positives" in per-individual tests by adopting a more conservative level of probability (P<.01) to indicate significant differences. Too much possibly useful information appeared to be discarded by reporting only those 7 or 9 of 88 parameters which significantly differed from control frequencies based on a Mann-Whitney U test. The reader is thus given the option of examining what are possibly borderline effects, and of examining the patterns of response revealed in the consideration of all or most of the parameters employed.

Indeed in order not to discard data, and since the overall pattern of response to treatment by the skeleton is of interest, two other ways were employed of dealing with the data. One of these consists of a graphic representation of all of the 67 characters which show variation of any sort in these experiments (Figure 1), in which histograms are presented comparing each experimental group separately with the untreated. Looking at all 67 variants, the deviation from UNTD in the negative groups (VEH, 245T-L) is randomly distributed in terms of number of characters the frequencies of which is increased or decreased, and the average deviation, taking direction into account, is almost precisely zero. The average absolute value of the deviation from untreated is 4%. In the positive control (245T-H) and Trifluralin there are many more characters increased in frequency than decreased. Most of this is accounted for by those characters which differ significantly. Among significantly affected variants 17 of 19 are increased in frequency in 245T-H relative to UNTD and the average absolute difference of all 19 is 23.7%. Ten of the 12 significantly affected traits in TFL are also increased relative to UNTD;

the average absolute difference here is 18.8%. Unaffected variants in these groups do deviate randomly as well as trivially from UNTD.

The second method is calculation of a single index number for each experimental group, based on the difference in frequency for each parameter which varies, regardless of significance level for each variant. The method, called a "Mean Divergence Estimate" (MDE) was devised by C.A.B. Smith at University College London, based on many of these same skeletal variants, in order to determine the degree of divergence of different sublines of the same inbred mouse strain. The method is described by Grewal ('62) and Berry ('63, '64), and is presented in the legend of Table 3. What this statistic provides is a measure of difference between any two populations based upon the frequencies of occurrence of a series of binomial characters. Specifically it presents an average squared difference corrected for inhomogeneities of variance by transformation of the frequencies, and also corrected for the magnifying effect of sampling variance. Thus the first line of Table 3 shows an average difference (MDE) between each Experimental group and Untreated for all 67 characters. The more similar the two groups being considered are, the closer will the MDE approach zero. The more dissimilar the two groups are (e.g. large treatment effects compared with untreated) the higher will be the value of the MDE. In this comparison the two negative groups are nearly identical with untreated (MDE-Vehicle = 0.022, 245T-L = 0.021), whereas the positive control (245T-H) was quite different (MDE = 0.148), and the Trifluralin group was somewhere between (MDE = 0.050). This method can be used to compare any two groups with one another. Thus the 245T-H group is seen (Table 3) to be equally different from all of the other groups, not just from Untreated. Indeed, by calculating an average MDE for all possible comparisons it is possible to obtain an index value of what can be considered a "Measure of Uniqueness" (Berry '63). These indices distill a large volume of data into a single number the magnitude of which is a measure of effect of treatment, which can be compared with other treatments within a series, and which allows utilization of all of the data obtained. These indices may also allow comparisons of different series of experiments. The MDE values obtained in comparisons of each Experimental group with untreated are consistent with the results of the other analyses performed.

All methods of analysis show that the known teratogen 2,4,5-T, in a teratogenic dose, is detectable by its effects upon the skeletons of animals which are classed as normal by the traditional criteria of teratogenesis studies. These animals, with no visible malformations, differ dramatically from untreated with respect to frequencies of occurrence of a sizeable number of variations in skeletal development. Even by the most conservative analysis (Table 2, Mann-Whitney U entries) the frontal bones of the skull and the cervical vertebrae are sensitive to the prenatal presence of teratogenic doses of 2,4,5-T. By contrast, a maximal "no-effect" level of this compound does not influence skeletal development to any significant extent. The principal contribution of this type of study lies in its ability to assess biological effects of developmental stress in the absence of gross abnormality. By examining the "vital statistics" of the experiment (Table 1) 2,4,5-T can be seen to be producing major effects. Thus just over one-fourth of treated females produce live born litters, and the average birth weight of liveborn pups is significantly reduced. Add that to the maternal mortality, nonproductive matings and the presence of stillborn litters, and the harmful effects of this dose level of 2,4,5-T are not in doubt. However, by looking retrospectively at a surviving population no members of which are abnormal it is still possible, by examining these skeletal variants, to demonstrate subtle effects of prenatal exposure to 2,4,5-T. And therein lies the strength of this assay system. It provides a means of examining a population of normal appearing individuals which have been exposed *in utero* to a potentially noxious agent, and to assess possible biological effects of that exposure.

Finally, in addition to quantitative effects of prenatal exposure we can also describe qualitative effects of treatment. Thus there are some specific variants which appear to be unique to the 245T-H group, at least one which is unique to TFL and, several which may be sensitive indicators of stress by virtue of being affected in both positive groups or even in the vehicle groups. One may eliminate solvent and handling effects by contrasting frequencies of occurrence in each compound treated group with those in the Vehicle-treated group. This comparison reveals several important effects in the 245T-H treated animals. The most prominent of these include the occurrence of Foramina Transversaria Imperfecta in both the first and second cervical vertebrae (Table 2 #32,34) in about half of the specimens in this group and very few UNTD or VEH. There are several other variants of the cervical vertebrae which are dramatically affected only in the 245T-H group. These include Dyssymphysis of the second cervical (#33), and a shift of the arch Foramina from the fourth to the fifth cervical vertebra (#36,37). Two other vertebral column effects peculiar to 245T-H are a loss of the prominent dorsal spine of the second thoracic vertebra (#46) which occurs in 45% of the compound-treated animals and none of the VEH treated animals, and a reduction in the number of caudal vertebrae.

In the skull, there is also a group of variants involving the frontal bones which is markedly increased in 245T-H. This group includes Fused Frontals (#10) and, also seen in Trifluralin-treated animals, a doubling of the frequency of occurrence of Parted Frontals (#9). From other series of experiments (Beck, unpublished data) Interfrontal and Parted Frontals appear often to be affected in this assay of skeletal variants as do also the Transverse Foramina of the first two cervical vertebrae. Thus these variants may be more generally indicative of prenatal stress than specific for this herbicide. This view is supported by the observation that, although the Frontal Bones of the skull of the Cervical vertebrae appear particularly responsive to 2,4,5-T exposure, they are, to a lesser extent also responsive to Trifluralin exposure as seen by increased frequency of Parted Frontals and the presence of Accessory Transverse Foramina in the cervical vertebrae (#39) in this latter group. Perhaps the most notable specific effects of the Trifluralin treatment is an increase in the frequency of occurrence of Fourteen Ribs (#54) and the single nature of the Foramen Ovale (#23, Figure 6).

From the results of this initial series of investigations it would seem that a Skeletal Variant Assay System such as is described here might well be used in detecting subtle biological effects of prenatal exposure to potentially noxious substances. The number of traits influenced, magnitude and direction of effects, and specific variants affected all provide useful information. Further series of experiments are under way using a variety of known teratogens (Trypan Blue, Acetazolamide, Bromodeoxyuridine, Cortisone) in order to better be able to calibrate the assay. Preliminary results from two replications of treatments with a series of trypan blue concentrations have been reported (Beck 1980). They showed close agreement with each other and with the results of the present study. The number of traits which varied were 66 and 58 and the number of variants which differed significantly from either UNTD or VEH was correlated with dose. At the teratogenic dose of trypan blue the only malformations seen at 62±2 days were tail kinks, there was no maternal toxicity, and there were 18 and 22 traits in the two series which differed significantly from UNTD, 24 and 17, respectively, which differed from vehicle-treated controls. Ideally, some correlations between known teratogenic effects from traditional types of investigations, with patterns of response of the skeletal variants, would lead to a predictive value for this type of assay when dealing with agents whose teratogenic properties are not known. At the least, this assay provides yet another avenue for postnatal assessment of prenatal exposures.

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FOOTNOTE

1. The work herein described was started while the author was on leave from Wheaton College on an Intergovernmental Personnel Act exchange, and was a visiting biologist at the United States Environmental Protection Agency, Health Effects Research Laboratory, Toxic Effects Branch - Environmental Toxicology Divison, at Research Triangle Park, N.C. It was completed with Federal funds from the Environmental Protection Agency under grant number R805420. The contents do not necessarily reflect the views and policies of the E.P.A., nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

Table 1. Vital statistics following ten daily intubations on days 6-15 of gestation, of 3.33 ml intubate per kg body weight per presentation.

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Parameter	Untreated Control	Corn Oil Control	2,4,5-T (20mg/kg)	2,4,5-T (100mg/kg)	Trifluralin (1.0g/kg)
No. Females Mated	25	25	25	29[a]	25
Died in Treatment	0	0	1	7	3
No Litter Produced	2	7	4	12	6
Stillborn Litters	0	0	0	2	3
No. Litters Born	23	18	20	8	13
Liveborn Pups (N)	243	174	178	81[e]	122
Atypical Live Pups	2	8	7	5	14
Stillborn Pups[b]	5	5	16	20[f]	18
Mating Efficiency (±SE) (No. Live Litters/No. Mated)[c]	.92±.05	.72±.09	.68±.09	.28±.08***	.52±.10***
Mean Litter Size (±SD) at Birth	10.6±2.4	9.7±3.6	8.9±3.8	10.1±2.9	9.4±2.7
Mean Birth Weight (g±SD)[d]	1.61±.10	1.59±.13	1.58±.13	1.45±.16*	1.58±.16
No. of Pups Weighed (N)	200	160	178	62	122
Frequency of Atypical Pups (%±SE)[c] (Atypical/Liveborn x 100)	0.8±0.6	4.6±1.6*	3.9±1.5*	6.2±2.7*	11.5±2.9***
Frequency Stillborn (%±SE)[c] (Stillborn/Liveborn + Stillborn x 100)	2.0±0.9	2.8±1.2	8.2±2.0**	19.8±4.0***	14.1±2.9***
Weaning Weight (at 28 DPN) Males (g±SD)	19.6±4.6	18.1±5.6	15.8±5.3	18.6±4.1	16.3±2.8
Females (g±SD)	15.2±3.6	15.8±4.6	14.5±4.5	16.4±2.4	14.9±2.5

Legend and Notes:

a. Additional females added to compensate for mortality during treatment
b. Stillbirths are under-represented since not all are detected
c. Tests of significance (t-tests) performed on arcsin-square root transformed frequencies, contrasting each experimental group with untreated.
d. Based on the litter as the experimental unit.
e. When it became clear that not enough pups would be produced to carry out the experiment a second round of treatments was performed. This treatment group, two months after the first, on the same batch of mice, gave poor mating efficiencies both in the 2,4,5-T treatment and in a Trifluralin treatment used as a control. No systematic analysis of vital statistics was attempted, but the 2,4,5-T treated animals produced were used for skeletal examinations.
f. Of the 20 stillborn pups, 19 were produced in two totally stillborn litters.

* = P<.05 ** = P<.01 *** = P<.001 when compared with untreated control-

Table 2. Frequencies of Occurrence of Skeletal Variants Among CD-1 Mice and Significant Differences in Frequencies Among Experimentals Contrasted with Untreated or Vehicle Treated Groups

				Frequencies (%)					Significant Differences									
									Compared With Untreated				Compared With Vehicle			Exclusive of Solvent Effects		
Character Number	Reference	Variant Number	Character Name N =	UNTD 94	VEH 95	245T-L 93	245T-H 95	TFL 92	VEH	245T-L	245T-H	TFL	245T-L	245T-H	TFL	245T-L	245T-H	TFL
1A	t	N	Short Snout															
2A	t	N	Bent Nose															
3D		N	Fused Nasals															
4D		N	Nasal-Turbinal Fusion															
5D		N	Nasal Fused to Skull															
6D	d	1.	Preorbital Foramen Double	44.7	50.5	52.7	28.4	43.5			o			*				
7D	a	2.	Preorbital Foramen Absent	1.1	1.1	0	5.3	0										
8D	c	3.	Maxillary Foramen I Double	94.7	94.7	83.9	94.7	93.5		o			o					
9D	c	4.	Maxillary Foramen I Absent	1.1	3.2	2.2	0	3.3						o				
10D	c	5.	Maxillary Foramen II Double	40.4	17.9	24.7	23.2	17.4	*	o	o	*†						
11D	c	6.	Maxillary Foramen II Absent	4.3	18.9	7.5	18.9	25.8	*		*	*	o					
12D	c	N	Lachrymal-Maxillary Fusion															
13D		N	Jugal Not Fused															
14A	v	7.	Interfrontal	3.2	8.4	3.2	20.0	6.5			*†			o				
15A	v	8.	I.F.-Frontal Fusion	0	2.1	1.1	2.1	0	o		o				o			
16A	v	9.	Parted Frontals	23.4	24.2	10.8	50.5	46.7		o	*†	*†	.o	*†	*		*†	*
17A	g	10.	Fused Frontals	0	0	0	13.7	1.1			*†			*†			*†	
18A	d	11.	Frontal Foramen Double	0	0	0	4.2	0			*			*			*	
19A	a	12.	Frontal Foramen Absent	6.4	3.2	2.2	5.3	6.5										
20A	a	13.	Accessory Parietal	1.1	3.2	2.2	8.4	4.3			*							
21D	d	14.	Foramen Palatinum Major Double	24.5	24.2	30.1	32.6	32.6										
22D	a	15.	Foramen Palatinum Minus Anterior Double	75.5	69.5	69.9	71.6	69.6										
23D	c	16.	Foramen Palatinum Minus Anterior Absent	8.5	10.5	5.4	23.2	15.2			*			o				
24D	a	17.	Foramen Palatinum Minus Posterior Double	1.1	1.1	1.1	0	13.0				*			*			*
25D	c	18.	Foramen Palatinum Minus Posterior Absent	8.5	9.5	7.5	4.3	1.1				*			*†			*

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Table 2. (continued)

26D	w	19.	Preoptic Sutures (Presphenoid)	13.8	30.5	19.4	46.3	31.5	*		*†	*†		o				
27D	w	20.	Abnormal Metoptic Roots (Presphenoid)	51.1	46.3	34.4	49.5	47.8	•	o								
28D	g	U	Pre-Basisphenoid Fusion	U														
29D	e	21.	Foramen Sphenoidale Mediale Present	57.4	48.4	50.4	45.3	63.0							o			
30D	e	22.	Posterior Border of the Palatine: Anteriad Suture	76.6	77.9	72.0	92.6	82.6			*			*			*	
31D		N	Squamosal-Frontal Fusion															
32D	u	N	Squamosal-Parietal Fusion															
33D	e	23.	Foramen Ovale Single	36.2	37.9	44.1	49.5	62.0				*†			*†			*
34D	e	N	Foramen Ovale Open Posteriorly															
35D	c	24.	Foramen Infra Ovale Double	36.2	22.3	23.9	45.7	28.4	o					*				
36D	a	25.	Foramen Infra Ovale Absent	1.1	1.1	0	6.3	1.1			o			o				
37D	c	26.	Foramen Pterygoideus Double	2.1	3.2	2.2	9.6	7.9			o							
38D	e	27.	Processus Pterygoideus Absent	0	1.1	1.1	0	1.1										
39D	u	N	Occipital-Periotic Fusion															
40D	g	N	Interparietal-Occipital Fusion															
41D	d	N	Basisphenoid-Basiooccipital Fusion															
42A	d	28.	Fenestra Flocculi Absent	5.3	12.6	9.7	7.4	15.2				o						
43D	e	29.	Foramina Hypoglossi Double (Occipital)	89.4	92.6	94.6	92.6	88.0										
44D	c	N	Mandibular Foramen Double															
45A	e	30.	Accessory Mental Foramen	4.3	10.5	8.6	10.5	8.7										
46A	i	31.	Dyssymphysis Atlas (C.1)	1.1	1.1	1.1	0	1.1					•					
47D	s	32.	Foramina Transversaria Imperfecta Atlas (C.1)	8.5	7.4	2.2	60.0	6.5		o	*†			*†			*†	
48A	i	33.	Dyssymphysis Axis (C.2)	3.2	3.2	2.2	28.4	4.3			*†			*†			*†	
49D	s	34.	Foramina Transversaria Imperfecta Axis (C.2)	3.2	1.1	11.8	48.4	1.1		o	*†		*	*†			*†	
50D	i	35.	Spinous Process C.3	0	0	0	0	2.2				o			o			
51A	u	36.	Arch Foramen C.4 Absent	31.9	40.0	15.1	6.3	19.6		*	*†		*†	*†	*†	*	*†	
52A	u	37.	Arch Foramen C.5 Present	6.4	3.2	7.5	22.8	2.2			*			*†			*	
53D	i	38.	Foramina Transversaria Imperfecta C.3-C.6	7.4	22.1	8.6	27.4	2.2	*		*		*		*†			

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Table 2. (continued)

54D	x	39.	Accessory Transverse Foramina C.3-C.6	57.4	49.5	59.1	60.0	78.3				*			*†	
55A	i	40.	Tubercula Anteriori C.6 Absent	1.1	0	0	1.1	0								
56A	i	41.	Tubercula Anteriori C.6 Inflexed	1.1	1.1	0	1.1	0								
57A	i	42.	Tubercula Anteriori C.6 Dystopic	0	0	1.1	1.1	0								
58A	d	U	Tubercula Anteriori C.6 Accessory					U								
59A	i,s	43.	Malformations (Dyss/Fus) C.3-C.7	0	0	2.2	0	2.2		o			o		o	
60D	d	44.	Foramina Transversaria C.7	54.3	52.6	64.1	56.8	45.7				o				
61A	i,t	45.	Malformations (Dyss/Fus) Th.1-Th.2	1.1	2.1	1.1	1.1	3.3								
62A	i	46.	Spinous Process Th.2 Absent	3.2	0	7.5	45.3	2.2	o		*†		*	*†	o	*†
63A	i	47.	Spinous Process Th.2 Reduced	0	6.3	2.2	10.5	6.5	*†	o	*	*†				
64A	a	48.	Spinous Process Th.2 Jointed	10.6	2.1	8.6	2.1	4.3	o		o		o			
65A	i	49.	Spinous Process Th.2 Dystopic	1.1	0	2.2	1.1	0					o			
66A	i	50.	Spinous Process Th.2 Accessory	0	2.1	0	0	2.2	o			o	o	o		
67A	s	51.	Arch Foramina Thoracic Vert.	75.5	64.2	78.5	66.3	70.7					o			
68A	p	52.	Reduced Articular Processes-Thoracic Vert.	10.6	13.5	24.7	14.7	27.2		o		*†			o	
69A	g	53.	Malformations (Dyss/Fus) Th.3-Th.14	0	3.2	2.2	1.1	2.2	o	o		o				
70A	h,r	54.	Fourteen Ribs	22.3	24.2	32.3	30.5	58.7				*†			*†	*†
71A	n	U	Malformed Ribs				U									
72A	k	55.	Fused Sternebrae	50.0	41.1	47.3	63.2	45.7						*		
73A	k	56.	Malformed Sternebrae	71.3	69.5	67.7	69.5	78.3								
74	x	57.	Fossa Olecrani Perforata (Humerus)	55.3	45.3	58.1	31.6	60.9			*				o	
75A	b,f,m	58.	Carpal Fusions	10.6	15.8	14.0	5.3	23.9				o		o		
76D	q,s	U	Sacralized L.6			U										

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Table 2. (continued)

77A		U	Lumbar Fusions/Malf		U													
78A	h	59.	27-Presacral Vertebrae	0	0	3.2	2.1	0		o	o		o	o				
79A	q,s	60.	Sacral Fusions Dorsal Processes	23.4	21.2	15.1	40.0	29.3			o			*				
80A		61.	Sacral Fusions Transverse Processes	1.1	0	0	0	1.1	•									
81A		62.	Sacral Fusions Vertebral Body	1.1	2.1	0	2.1	4.3										
82A	q	63.	Caudalized S.3	3.2	18.9	17.2	3.2	6.5	*	*				*	*			
83D	k	64.	Foramina Acetabuli (Innominate)	31.9	28.4	35.5	32.6	33.7										
84D	i,k	65.	Dyssymphysis Ischio-Pubica	1.1	1.1	2.2	3.2	2.2										
85D	d	N	Pubic Fusion															
86A	b,f,m	N	Tarsal Fusion															
87A		66.	Fewer Than 30 Caudal Vert.	19.1	25.3	36.6	58.5	42.4		*	*	*		*	*		*	*
88A		67.	Caudal Fusions	1.1	1.1	0	0	1.1										
			Number of Significant Differences					t-test	6	3	19	12	4	16	10	1	11	7
								Mann-Whitney U	1	0	9	7	1	8	6	0	7	2

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Legend for Table 2.

Character Number = An anterior-posterior listing of the 88 characters investigated. A = Examined in articulated skeletons; D = Examined in disarticulated skeletons

Reference - Sources of description of each character

a. New Variant, described here
b. Beck and Smith '72
c. Berry '63
d. Berry and Searle '63
e. Deol '55
f. Deol '61
g. Deol and Trusolve '57
h. Green '41
i. Grüneberg '50
j. Grüneberg '51
k. Grüneberg '52
m. Grüneberg '56
n. Grüneberg '63
p. Johnson '68
q. Runner '53
r. Palmer '72
s. Searle '54a
t. Searle '54b
u. Searle '54c
v. Truslove '52
w. Truslove '54
x. Weber '50

Variant Number = An anterior-posterior listing of those character which vary in these experiments. Variants are defined as characters which occur in more than one of the 469 individuals examined. N = Null class - character not seen in any specimen; U = Unique observation - character seen in a single specimen. The letter also appears in the column in which the unique observation occurred.

Character Name = Names used to describe characters. Most are the terminology of Bateman (1954), Berry (1963), or are described in the text.
C = Cervical, Th = Thoracic, L = Lumbar, S = Sacral Vertebrae
Dyss = Dyssymphysis
Fus = Fusion
Vert = Vertebrae, Malf = Malformation

N = Number of Specimens in each group

Frequencies = Percentage of specimens in each group which manifest each variant.

UNTD = Untreated, unhandled controls

VEH = Corn oil vehicle control. Vehicle control dams were intubated intragastrically with 3.3 ml/kg body weight of corn oil on days 6-15 of gestation.

245T-L = Low dose (negative control) of 2,4,5-T. Dams were intubated with 3.3 mg/kg/day of corn oil containing 20 mg/kg/treatment of 2,4,5-T on days 6-15 of gestation.

245-H = High dose (positive control) of 2,4,5-T. Dams were intubated with 100 mg 2,4,5-T/kg body weight/days 6-15 of gestation in 3.3 ml/kg/treatment of corn oil

TFL =Trifluralin (unknown compound). Dams were intubated with 1.0 gm Trifluralin/kg body weight in 3.3 ml/kg corn oil, warmed to 38-40°C, on each of days 6-15 of gestation.

Significant Differences

Tests of significance, contrasting each treatment group with UNTD, and each compound-treated group with VEH (the UNTD-VEH comparison is identical with a VEH-UNTD comparison and is not repeated). The statistical analysis includes t-tests on arcsin-square root transformed frequencies (Sokal and Rohlf 1969, pp. 607-610) where $t = \arcsin\sqrt{p_1} - \arcsin\sqrt{p_2} / \sqrt{820.8(1/n_1 + 1/n_2)}$, significance being defined as $P < .01$, and the individual mouse is the experimental unit. * = $P < .01$; o = $P < .05$, presented for completeness. Also represented in this group of columns are the results of Mann-Whitney U tests (Siegal, '56, pp. 116 cf) in which the litter is the experimental unit, litter percents for the two groups being contrasted are ranked for each character compared. This conservative non-parametric test was only performed on data for which t-tests yielded significant differences. Significance on the Mann-Whitney U test was defined at the $P < .05$ level († = $P < .05$).

Variants were said to show significant differences in compound-treated animals exclusive of any solvent or vehicle effects when they differed significantly from both VEH and UNTD. These are listed in the last three columns of the table for clarity.

Table 3. Mean Divergence Estimates

	VEH	245T-L	245T-H	TFL	Measure of Uniqueness
UNTD	0.0219	0.0207	0.1481	0.0505	.0603
VEH		0.0255	0.1526	0.0393	.0598
245T-L			0.1476	0.0466	.0601
245T-H				0.1485	.1492
TFL					.0712

Legend: The mean divergence estimate (MDE) is an average for all 67 characters of the difference in frequency between the two groups being compared.

$$MDE = \Sigma\ [(\theta_1 - \theta_2)^2 - (1/n_1 + 1/n_2)]/N$$

where

$\theta = \sin^{-1}(1-2p)$ in radians. This transformation makes sampling variance independent of the frequency of the character

p = frequency of occurrence of character

n = number of individuals in sample

Σ = sum for all characters (i.e. 67)

N = number of characters being considered (i.e. 67)

Note that the sampling variance of $\theta = 1/n$

$(\theta_1 - \theta_2)^2$ = measure of divergence or difference in frequency between two groups for each character

$(1/n_1 + 1/n_2)$ = variance of $\theta1 - \theta2$ due to sampling. this value inflates $\theta_1 - \theta_2$ and is therefore subtracted.

Measure of Uniqueness = an average MDE for all comparisons involving one group.

See Grewal (1962) and Berry (1963, 1964) for additional details and text for discussion of these indices.

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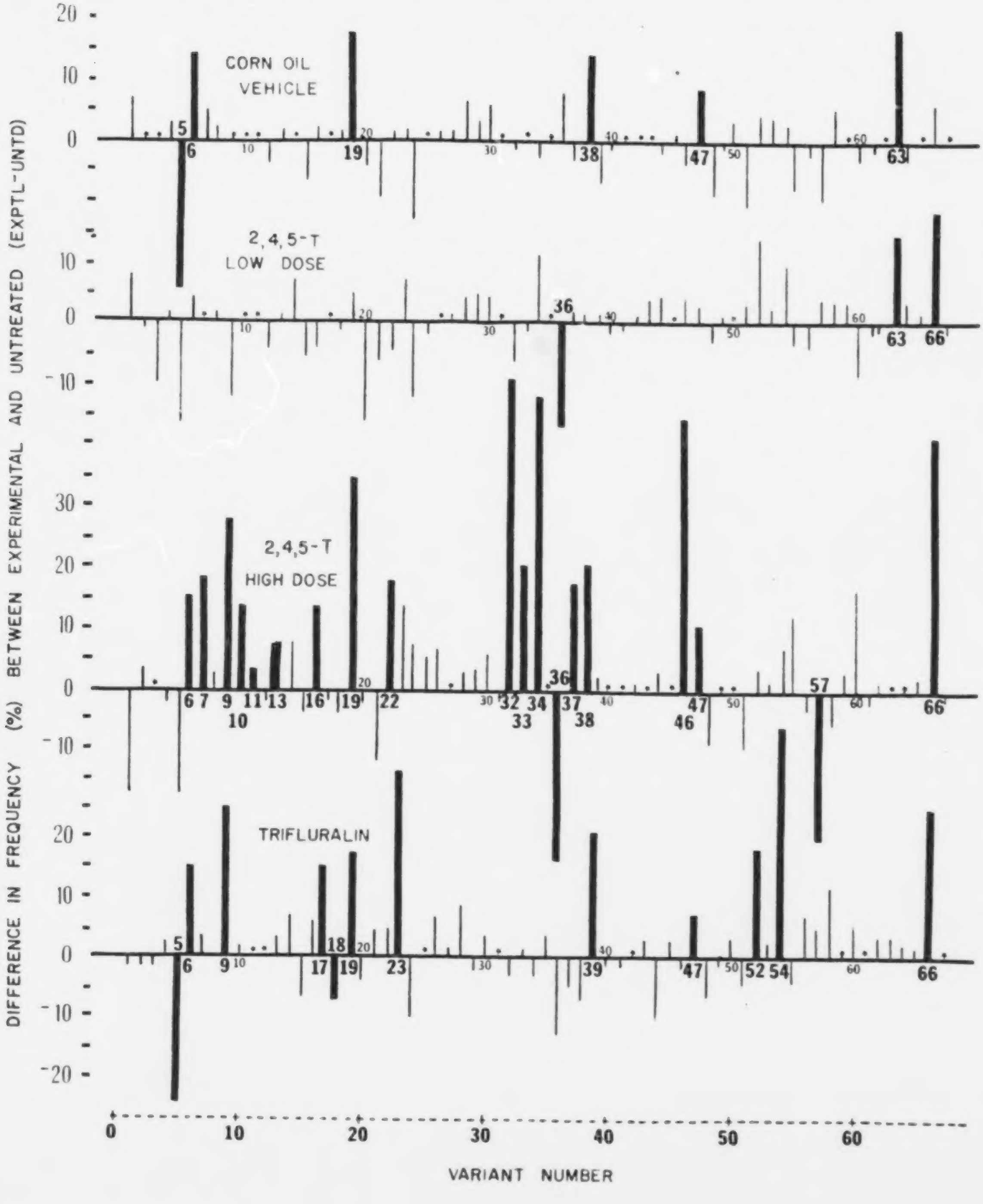
Figure 1. Percent Difference in Frequency of Occurrence of Each Variant Between Each Experiment Group and Untreated.

Legend:

Values above the baseline are those in which the frequency (animals showing variant/number in sample x 100) was higher in the experimental than in Untreated; values below the base line represent a decreased frequency of occurrence in experimentals relative to untreated.

Heavy bars indicate that the difference between experimental and Untreated is significant ($P<.01$ from the results of t-tests of arcsin square-root transformed frequencies)


DIFFERENCE IN FREQUENCY (%) BETWEEN EXPERIMENTAL AND UNTREATED (EXPTL-UNTD)
CORN OIL VEHICLE
20
10
0
5
6
19
38
47
63
2,4,5-T LOW DOSE
10
0
-10
36
63
66
2,4,5-T HIGH DOSE
30
20
10
0
-10
6 7 9 10 11 13 16 19 22 32 33 34 36 37 38 46 47 57 66
TRIFLURALIN
20
10
0
-10
-20
5 6 9 17 18 19 23 39 47 52 54 66
0
10
20
30
40
50
60
VARIANT NUMBER

Legend for Plate 1

Figure 2. Dorsal view of a disarticulated vehicle-treated specimen showing presence of an Interfrontal bone (7) as a separate entity between the two Frontal bones. The Frontals are not parted, nor do they show any fusion along the dorsal midline suture.

Figure 3. Dorsal view of a disarticulated vehicle-treated specimen showing Parted Frontals (9). There is no Interfrontal present, nor any fusion of mid-dorsal suture.

Figure 4. Dorsal view of a disarticulated specimen from a different experimental series showing Accessory Parietal bones (13) bilaterally expressed. When present these bones are completely delimited by a suture from the Parietal bones in the anterior-medial region. Expression may also be unilateral.

Figure 5. Left lateral view of a disarticulated specimen treated with 2,4,5-T at the high dose level, showing a single Preorbital Foramen (1A) and a single Frontal Foramen (11A). The variants in each case involve a doubling of the foramen.

Figure 6. Posterior view of an anterior portion of the skull from a disarticulated specimen treated with the high dose of 2,4,5-T showing the following structures:

19A Region of the Presphenoid bone in which Preoptic sutures, when present, occur
20 Abnormal Metoptic Root of the Ala Orbitalis of the Presphenoid bone. The Metoptic Root on the contralateral side is normal.
21 Foramen Mediale present in the Basisphenoid bone
23 Single Foramen Ovale in the Sphenoid bone
23A Doubled Foramen Ovale
24 Region of the Sphenoid in which the Foramen Infra Ovale occurs

Legend for Plate 2

Figure 7. Dorsal view of the cervical region of an articulated vehicle-treated specimen.

C1-C5 The first five Cervical Vertebrae. Note that the transverse foramina of C1 are complete; the spinous process and neural arch of C2 are typical in appearance; arch foramina of C3 present a typical appearance as well.

36A Arch Foramen of C4; the variant form of this character involves absence of the foramen.

37A There is no arch foramen on C5. The variant form involves presence of a foramen on one or both sides. Animals may show arch foramina on both C4 and C5.

Figure 8. Dorsal view of the cervical region of an articulated specimen from a different series showing Foramina Transversaria Imperfecta of C1 (32). Also discernable are mild dyssymphyses in the neural arches of C5 and C6 (43).

Figure 9. Dorsal view of the cervical region of an articulated specimen showing one type of dyssymphysis of C2 (33). Gaps in the midline between the two halves of the neural arch, absence of the spinous process, and more obvious malformations are also seen. Because of the absence of the spinous process of C2 one can see that there is no Spinous Process on C3 (35A). Usually this character is examined in disarticulated specimens. This specimen also shows absence of the arch foramen in C4 (36).

Figure 10. Posterior oblique view of the second Cervical Vertebra (C2) showing intact Transverse Foramina (34A).

Figure 11. Posterior oblique view of the second cervical vertebra from a specimen which was treated with the high dose of 2,4,5-T, showing Imperfect Transverse Foramina (34).

Figure 12. Posterior view of the sixth Cervical Vertebra (C6) from a specimen treated with the high dose of 2,4,5-T showing an Accessory Transverse Foramen on the left side (39), the usual appearance of the Transverse Foramen on the right (38A) with no accessory foramen, and the usual appearance of the Tubercula Anteriori (40A) of C6.

Legend for Plate 3.

Figure 13. Right lateral view of the articulated cervico-thoracic border of an animal treated with a high dose of 2,4,5-T, showing the last cervical (C7) and first four thoracic vertebrae (the first three are labeled T1-T3). The prominent Processus Spinosus of T2 is seen (46A) showing no variants.

Figure 14. Right lateral view of the articulated fifth cervical through fourth thoracic vertebrae of an animal treated with the low dose of 2,4,5-T, showing a Jointed Processus Spinosus (48) of T2.

Figure 15. Dorsal view of the articulated cervico-thoracic border of a specimen treated with the high dose of 2,4,5-T. The seventh cervical and first three thoracic vertebrae are labeled C7, and T1-T3 respectively. There is absence of the Processus Spinosus of T2 (46).

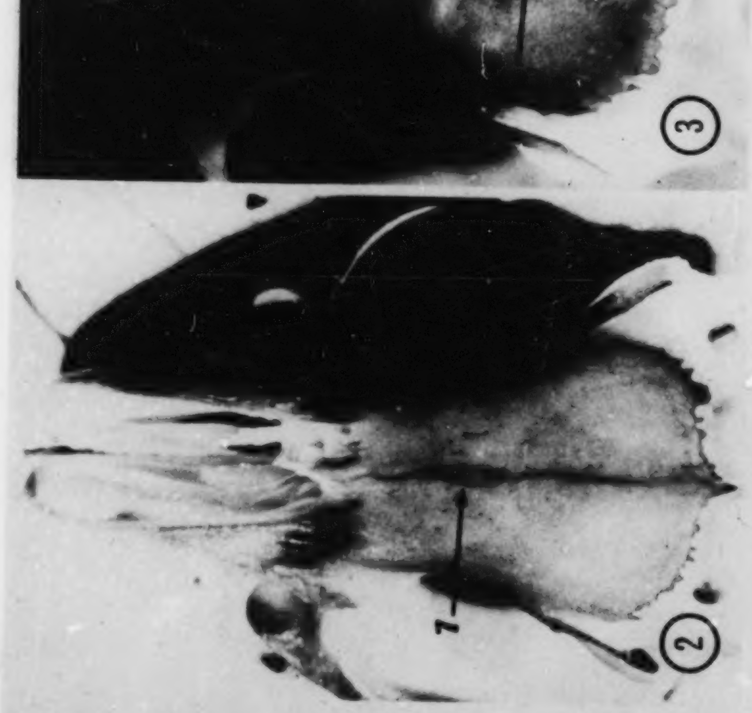
Figure 16. A dorsal view of the articulated thoracico-lumbar border of a vehicle-treated specimen showing a fourteenth rib on the left (54) and none on the right.

Figure 17. Dorsal view of the articulated hind feet of a specimen from a different series in which the second and third tarsal bones (tar-2, tar-3) are fused in the left foot (LH) and not in the right (RH). cen=centrale

Figure 18. Dorsal view of the articulated left front foot (LF) of an animal treated with the high dose of 2,4,5-T, showing fusion of the second carpal (car-2) with the centrale (cen). The first and third carpals (car-1, car-3) are separate, and the fourth and fifth carpals (car 4/5) are invariably fused.

Figure 19. Dorsal view of articulated right front foot (RF) of an animal from a different series showing no fusions among the carpal bones (car 1-3, cen) except for the invariate fusion of car 4/5.

PLATE 1.



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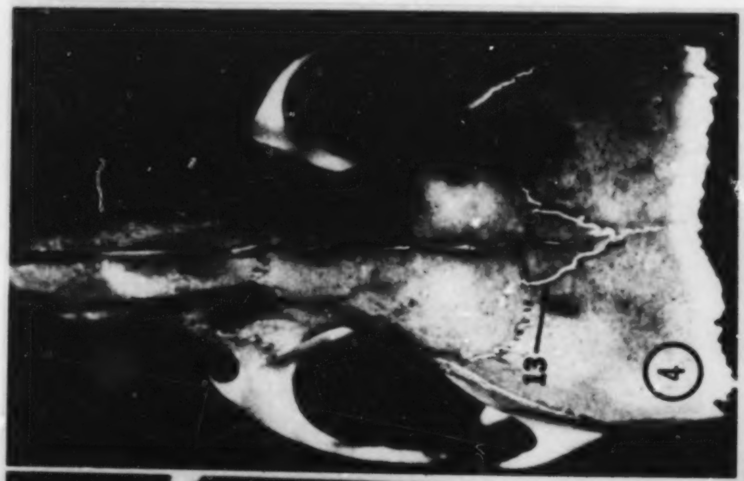

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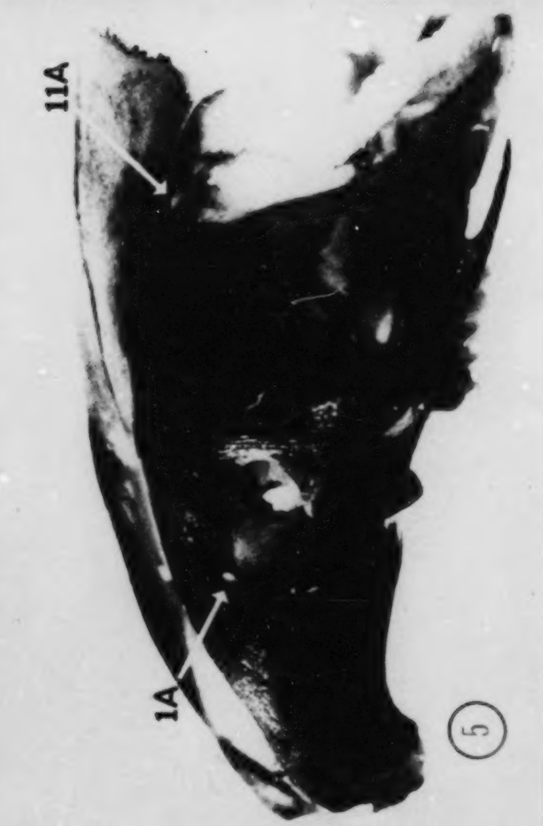

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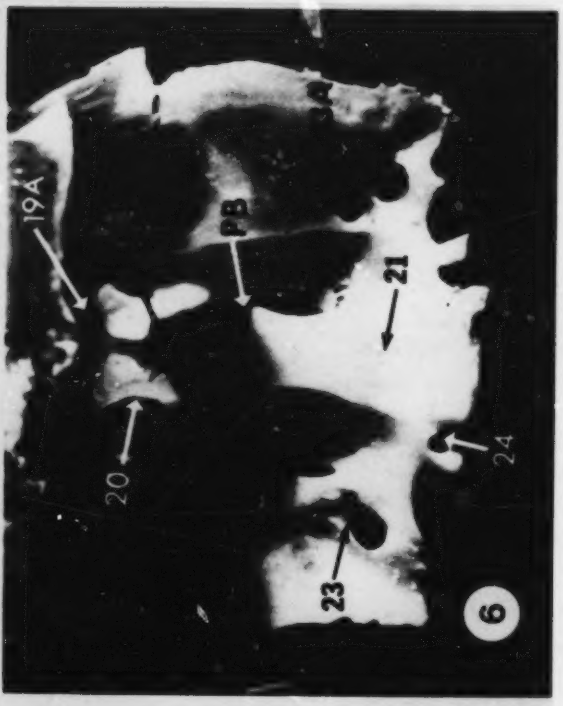
19A
PB
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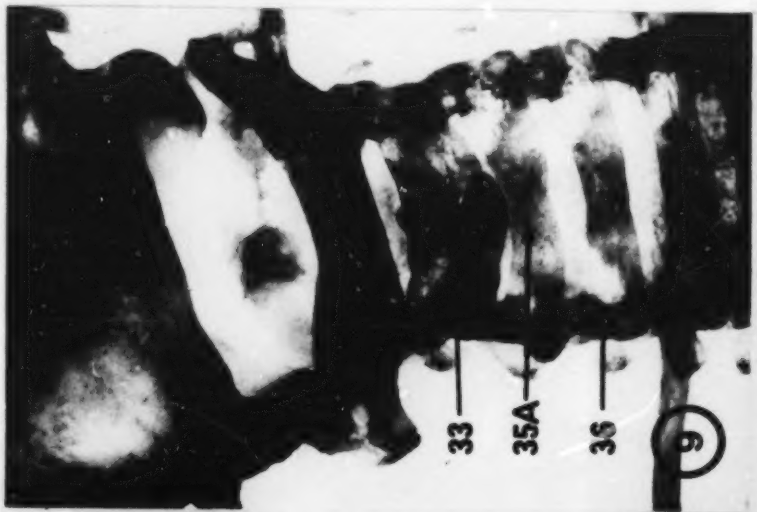
PLATE 2.



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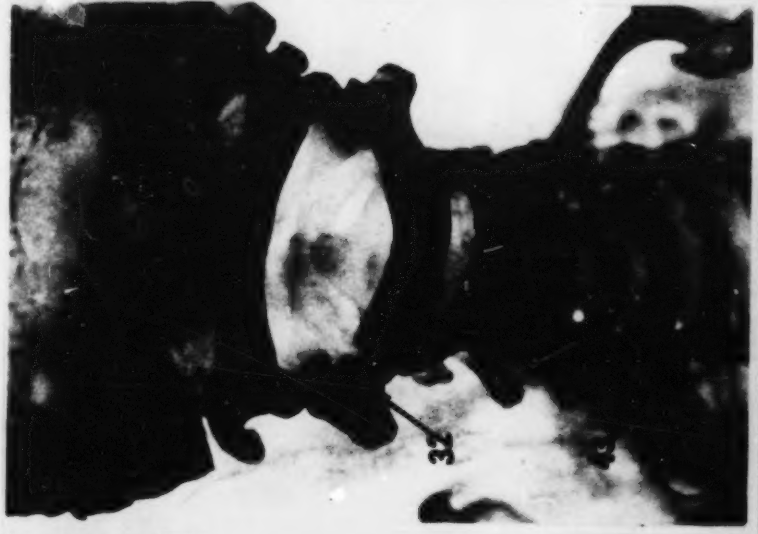
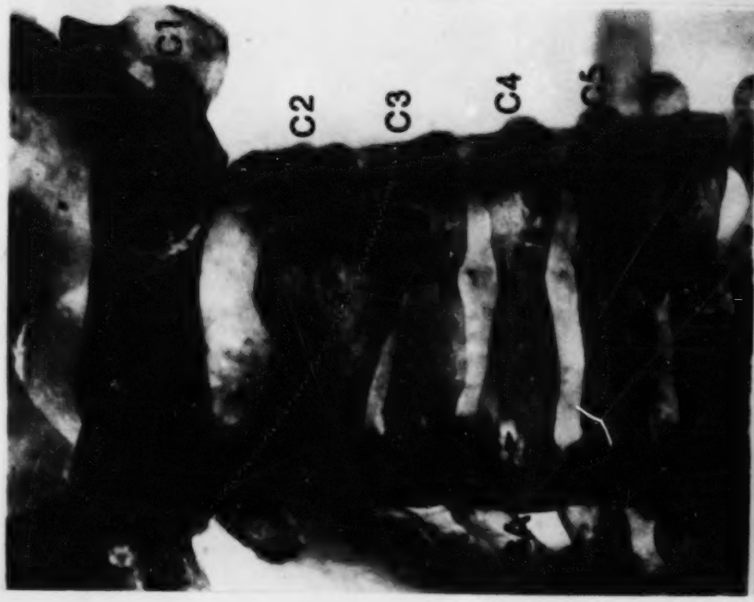
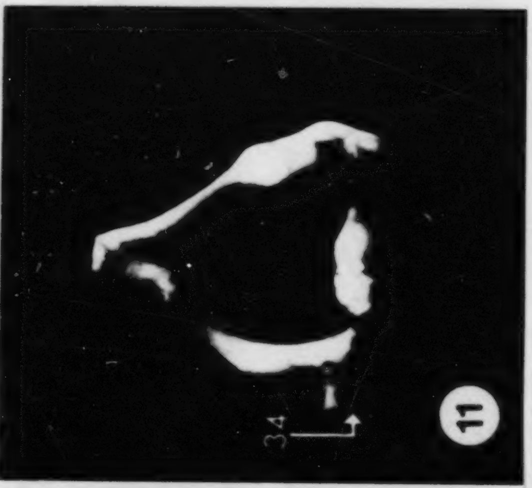



C1
C2
C3
C4
C5



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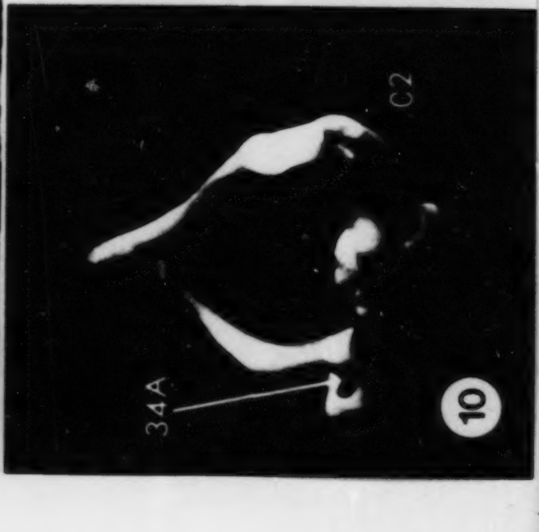

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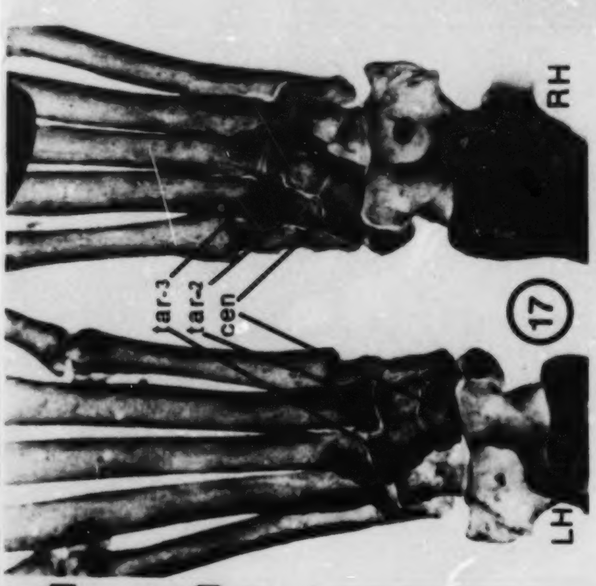
PLATE 3.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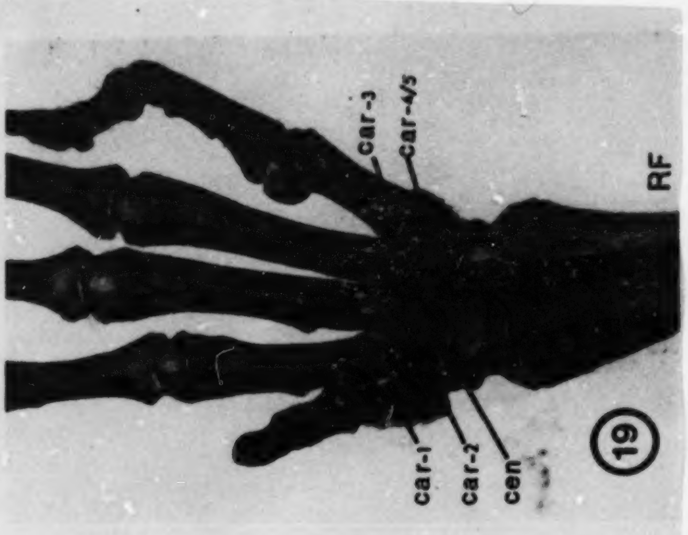
LH
RH
tar-3
tar-2
cen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RF
car-1
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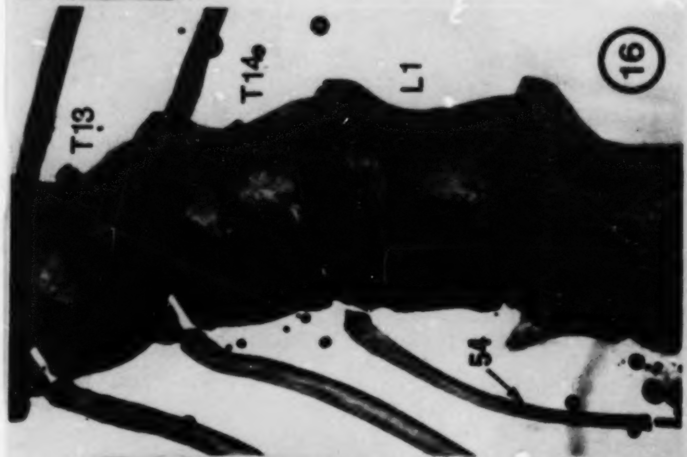
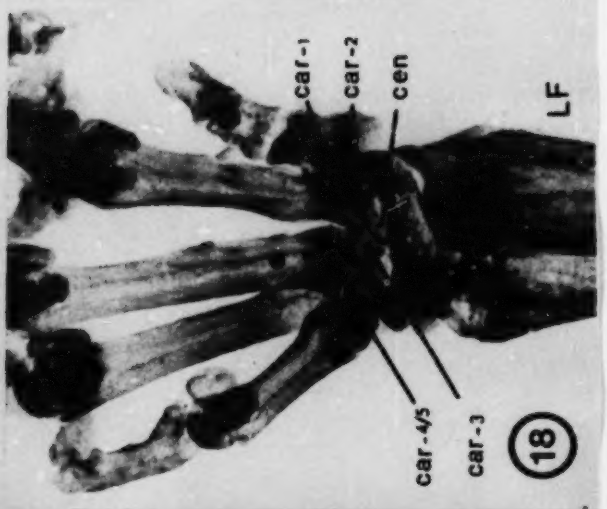
car-4/5


⑯
T13
T14
L1
54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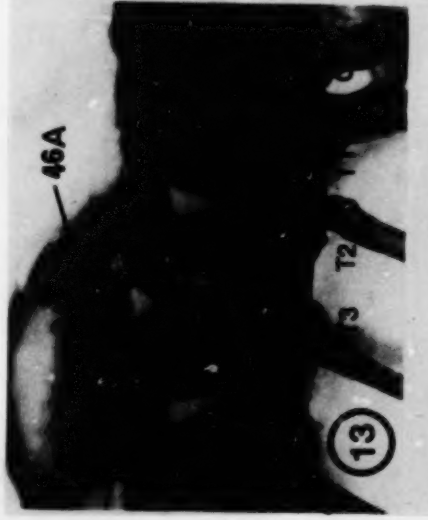
LF
car-1
car-2
cen
car-4/5
car-3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T2
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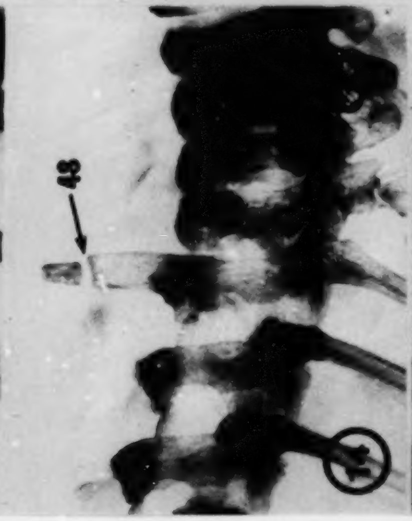
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Teratology Society Meeting 1981

E6. **ABSTRACT REPRODUCTION FORM**

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BECK, S.L., Department of Biology, Wheaton College, Norton, Massachusetts 02766

Postnatal effects on the CD-1 mouse skeleton of prenatal exposure to acetazolamide.

Two sets of experiments were performed (I, II) with s.c. injection at 7AM, 3PM, and 12M, of a total of 0, 200mg (L), or 1.0gm (H) acetazolamide/kg in 10ml H_2O/kg on D8 or D9-11 of gestation. Ectrodactyly occurred only in D9-11 H. Tail kinks were common in D9-11 L and H in I and II at sacrifice. Skeletons were examined at 62±2 DPN for 88 all-or-none variants, and a novel variant, Frontal Extension. Frequencies were contrasted with untreated (UNTD) and vehicle-treated (VEH) groups. Variation occurred in 62 and 67 characters in I and II. The number of variants which differed ($P<.01$) compared with UNTD and VEH was dose-related in both regimes in I and II. Agreement between the series was good, especially in D9-11 treatments. In D9-11 H, 13 variants differed from UNTD in I and II, 2 differed in I only and 5 in II only. In D8 H, 8 variants differed from UNTD in I and II, 4 in I only and 9 in II only. Variants which differed from UNTD and VEH in both I and II included Parted Frontals, Frontal Extension, Accessory Parietal, Preoptic Sutures, Abnormal Metoptic Roots, 27 Presacral Vertebrae, in D8; Foramen Ovale Single, Accessory Mental Foramen, Foramina Transversaria Imperfecta of the Atlas, Fused Sternebrae, Fossa Olecrani Perforata, Sacral Fusions- Transverse Processes and Vertebral Body, Fewer than 30 Caudal Vertebrae, and Caudal Fusions/Malformations, in D9-11; and Fourteen Ribs, Lumbar Fusions/Malformations, Sacral Fusions-Dorsal Processes, in both D8 and D9-11. A substantial amount of cryptic variation was detected in these experiments, and adds weight to the potential usefulness of this procedure as a screen for teratogenicity testing. (Supported by Research Grant R805420 from the U.S. Environmental Protection Agency.)

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If your material can be presented only from the platform, please document in accompanying letter.

☒ Poster Session

(Do not fold this sheet. Use cardboard backing to avoid damage in mail.)